

NOVEL IL-9/IL-2 RECEPTOR-LIKE MOLECULES
AND USES THEREOF

CROSS-REFERENCE TO RELATED APPLICATIONS

This application is a continuation-in-part application of copending U.S. Patent Application No. 09/313,913, filed May 18, 1999, , and entitled "Novel IL-9/IL-2
5 Receptor-Like Molecules and Uses Thereof," which is hereby incorporated herein in its entirety by reference.

FIELD OF THE INVENTION

The invention relates to novel IL-9/IL-2 receptor-like nucleic acid sequences and
10 proteins. Also provided are vectors, host cells, and recombinant methods for making and using the novel molecules.

BACKGROUND OF THE INVENTION

15 Critical interactions among cells of the immune system are controlled by mediators called cytokines. A diverse group of cytokines that are structurally dissimilar and genetically unrelated have been identified. The cytokines serve as crucial intercellular-signaling molecules that are responsible for the multidirectional communication among immune and inflammatory cells engaged in host defense, repair,
20 and restoration of homeostasis, as well as among other somatic cells in the connective tissues, skin, nervous system, and other organs. More particularly, this diverse group of intercellular-signaling proteins regulates local and systemic immune and inflammatory responses as well as wound healing, hematopoiesis, and many other biological processes. Each cytokine is secreted by particular cell types in response to a variety of stimuli and
25 produces a characteristic constellation of effects on the growth, motility, differentiation, or function of its target cells. In fact, cytokines regulate one another's production and

activities. Other types of biological mediators, such as corticosteroids and prostaglandins, have agonistic or antagonistic effects on cytokine activities.

Interleukin-2 (IL-2) is an autocrine and paracrine growth factor that is secreted by activated T lymphocytes. IL-2 is a critical immunoregulatory cytokine as it is essential
5 for clonal T-cell proliferation, is involved in cytokine production, and influences the functional properties of B cells, macrophages, and NK cells. IL-2 enhances proliferation and antibody secretion by normal B cells. However, the concentration required for the B-cell response is two- to three-fold higher than is required to obtain T-cell responses. Higher concentrations of IL-2 can also activate neutrophils. IL-2 exhibits a short half-life
10 in the circulation. Thus, it generally acts only on the cell that secreted it or on cells in the immediate vicinity.

The IL-2 molecule is a 15,400 molecular weight polypeptide having 133 amino acids. The molecule is encoded by a gene on human chromosome 4. The encoded protein is composed of two α helices arranged to form hydrophobic planar faces around a
15 very hydrophobic core.

The IL-2 receptor is not expressed in resting T cells but is induced to maximal levels within two or three days after the cells become activated. A decline in receptor expression occurs up to 6-10 days after activation. This transient nature of IL-2 receptor expression maintains the cyclical, self-limiting pattern of normal T-cell growth *in vivo*.
20

Interleukin-9 (IL-9) is a cytokine produced by activated Th2 cells upon stimulation with antigen. The cDNA sequence has been cloned. See, Wang *et al.* (1989) *Blood* 74:1880-1884. Both human and murine protein sequences contain 144 residues with a signal peptide of 18 amino acids. IL-9 expression seems to be mainly restricted to activated T cells.

IL-9 is capable of inducing the proliferation of mast cell lines, without the need for any additional growth factors. Besides this growth promoting activity, IL-9 may play a key role in mast cell differentiation by regulating the expression of mast cell proteases. IL-9 plays a role in Ig-mediated responses as suggested by the involvement of IL-9 in mast cell activation and proliferation, as well as the IL-9 production during parasite
30 infections.

A variety of mouse hemopoietic cells, including T cells, mast cells, and macrophages, express high affinity receptors for IL-9. The murine receptor contains 468 amino acids including an extracellular domain, comprising 233 amino acids that shows the typical features of the hematopoietin receptor superfamily. The human IL-9 receptor cDNA encodes a 522 amino acid protein with 53% identity to the murine IL-9 receptor.

During the course of an immune response, T cells differentiate into Th phenotypes defined by their pattern of cytokine secretion and immunomodulatory properties (Abbas *et al.* (1996) *Nature* 383:787). Th cells are composed of at least two distinct subpopulations, termed Th1 and Th2 cell subpopulations (Mosmann *et al.* (1989) *Ann. Rev. Immunol.* 7:145; Del Prete *et al.* (1991) *J. Clin. Invest.* 88:346; Wiernenga *et al.* (1990) *J. Immunol.* 144:4651; Yamamura *et al.* (1991) *Science* 254:277; Robinson *et al.* (1993) *J. Allergy Clin. Immunol.* 92:313). Th1 and Th2 cells appear to function as part of the different effector functions of the immune system (Mosmann *et al.* (1989) *Ann. Rev. Immunol.* 7:145). Specifically, Th1 cells direct the development of cell-mediated immunity, triggering phagocyte-mediated host defenses, and are associated with delayed hypersensitivity. Accordingly, infections with intracellular microbes tend to induce Th1-type responses. Th2 cells drive humoral immune responses, which are associated with, for example, defenses against certain helminthic parasites, and are involved in antibody and allergic responses.

Th1 cells secrete interleukin-2 (IL-2), interferon- γ (IFN- γ), and tumor necrosis factor- α (TNF- α). These cytokines enhance inflammatory cell-mediated responses and have a pathogenic role in the development of autoimmune disease. Th2 cells secrete interleukin-4 (IL-4), interleukin-5 (IL-5), interleukin-10 (IL-10), and interleukin-13 (IL-13). These cytokines suppress inflammatory responses while potentiating humoral immunity and control and reverse disease evolution (Scott *et al.* (1994) *Immunity* 1:73; Smith *et al.* (1998) *J. Immunol.* 160:4841; Abbas *et al.* (1996) *Nature* 383:787). The different type of cytokines released upon stimulation has been demonstrated to be central to disease evolution (Chu and Londei (1996) *J. Immunol.* 157:2685; Hsieh *et al.* (1993) *Science* 260:547).

The profile of the natural immune response, specifically cytokine production by natural killer cells or cells of basophil lineage, may determine the phenotype of the

subsequent immune response. Therefore, methods are needed to regulate an immune response.

SUMMARY OF THE INVENTION

5

Isolated nucleic acid molecules corresponding to IL-9/IL-2 receptor-like nucleic acid sequences are provided. Additionally, amino acid sequences corresponding to the polynucleotides are encompassed. In particular, the present invention provides for isolated nucleic acid molecules comprising nucleotide sequences encoding the amino acid
10 sequences shown in SEQ ID NO:2 and SEQ ID NO:4 or the nucleotide sequences encoding the DNA sequence deposited in a bacterial host as ATCC Accession Number _____. Further provided are IL-9/IL-2 receptor-like polypeptides having an amino acid sequence encoded by a nucleic acid molecule described herein.

The present invention also provides vectors and host cells for recombinant
15 expression of the nucleic acid molecules described herein, as well as methods of making such vectors and host cells and for using them for production of the polypeptides or peptides of the invention by recombinant techniques.

The IL-9/IL-2 receptor-like molecules of the present invention are useful for modulating the immune, inflammatory, and respiratory responses. The molecules are
20 useful for the diagnosis and treatment of immune and respiratory disorders, particularly for the treatment and diagnosis of T-lymphocyte-related disorders, including, but not limited to, atopic conditions, such as asthma and allergy, including allergic rhinitis, psoriasis, the effects of pathogen infection, chronic inflammatory diseases, organ-specific autoimmunity, graft rejection, and graft versus host disease. Additionally, the molecules
25 of the invention are useful as modulating agents in a variety of cellular processes including growth promoting activity, particularly the antigen-independent proliferation of T helper cell clones, and direct effects on normal hemopoietic progenitors, human T cells, B cells, thymocytes, thymic lymphomas, and neuronal cell lines. Accordingly, in one aspect, this invention provides isolated nucleic acid molecules encoding IL-9/IL-2
30 receptor-like proteins or biologically active portions thereof, as well as nucleic acid

fragments suitable as primers or hybridization probes for the detection of IL-9/IL-2 receptor-like-encoding nucleic acids.

Another aspect of this invention features isolated or recombinant IL-9/IL-2 receptor-like proteins and polypeptides. Preferred IL-9/IL-2 receptor-like proteins and polypeptides possess at least one biological activity possessed by naturally occurring IL-9/IL-2 receptor-like proteins.

Variant nucleic acid molecules and polypeptides substantially homologous to the nucleotide and amino acid sequences set forth in the sequence listings are encompassed by the present invention. Additionally, fragments and substantially homologous fragments of the nucleotide and amino acid sequences are provided.

Antibodies and antibody fragments that selectively bind the IL-9/IL-2 receptor-like polypeptides and fragments are provided. Such antibodies are useful in detecting the IL-9/IL-2 receptor-like polypeptides as well as in regulating the T-cell immune response and cellular activity, particularly growth and proliferation.

In another aspect, the present invention provides a method for detecting the presence of IL-9/IL-2 receptor-like activity or expression in a biological sample by contacting the biological sample with an agent capable of detecting an indicator of IL-9/IL-2 receptor-like activity such that the presence of IL-9/IL-2 receptor-like activity is detected in the biological sample.

In yet another aspect, the invention provides a method for modulating IL-9/IL-2 receptor-like activity comprising contacting a cell with an agent that modulates (inhibits or stimulates) IL-9/IL-2 receptor-like activity or expression such that IL-9/IL-2 receptor-like activity or expression in the cell is modulated. In one embodiment, the agent is an antibody that specifically binds to IL-9/IL-2 receptor-like protein. In another embodiment, the agent modulates expression of IL-9/IL-2 receptor-like protein by modulating transcription of an IL-9/IL-2 receptor-like gene, splicing of an IL-9/IL-2 receptor-like mRNA, or translation of an IL-9/IL-2 receptor-like mRNA. In yet another embodiment, the agent is a nucleic acid molecule having a nucleotide sequence that is antisense to the coding strand of the IL-9/IL-2 receptor-like mRNA or the IL-9/IL-2 receptor-like gene.

In one embodiment, the methods of the present invention are used to treat a subject having a disorder characterized by aberrant IL-9/IL-2 receptor-like protein activity or nucleic acid expression by administering an agent that is an IL-9/IL-2 receptor-like modulator to the subject. In one embodiment, the IL-9/IL-2 receptor-like modulator is an IL-9/IL-2 receptor-like protein. In another embodiment, the IL-9/IL-2 receptor-like modulator is an IL-9/IL-2 receptor-like nucleic acid molecule. In other embodiments, the IL-9/IL-2 receptor-like modulator is a peptide, peptidomimetic, or other small molecule.

The present invention also provides a diagnostic assay for identifying the presence or absence of a genetic lesion or mutation characterized by at least one of the following: (1) aberrant modification or mutation of a gene encoding an IL-9/IL-2 receptor-like protein; (2) misregulation of a gene encoding an IL-9/IL-2 receptor-like protein; and (3) aberrant post-translational modification of an IL-9/IL-2 receptor-like protein, wherein a wild-type form of the gene encodes a protein with an IL-9/IL-2 receptor-like activity.

In another aspect, the invention provides a method for identifying a compound that binds to or modulates the activity of an IL-9/IL-2 receptor-like protein. In general, such methods entail measuring a biological activity of an IL-9/IL-2 receptor-like protein in the presence and absence of a test compound and identifying those compounds that alter the activity of the IL-9/IL-2 receptor-like protein.

The invention also features methods for identifying a compound that modulates the expression of IL-9/IL-2 receptor-like genes by measuring the expression of the IL-9/IL-2 receptor-like sequences in the presence and absence of the compound.

Other features and advantages of the invention will be apparent from the following detailed description and claims.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows the amino acid sequence alignment for the human and murine 16445 proteins (SEQ ID NO:2 and SEQ ID NO:4, respectively) encoded by human 16445 (SEQ ID NO:1) and its murine orthologue 16445 (SEQ ID NO:3) with the human

Sub
A1
cont

IL-2 receptor beta chain (hIL-2Rb; SP Accession Number P14784; SEQ ID NO:5), murine IL-2 receptor beta chain (mIL-2Rb; SP Accession Number P16297; SEQ ID NO:6), human IL-9 receptor (hIL-9R; SP Accession Number Q01113; SEQ ID NO:7), and murine IL-9 receptor (mIL-9R; SP Accession Number Q01114; SEQ ID NO:8). The
5 sequence alignment was generated using the Clustal method.

The human and murine 16445 protein sequences share approximately 64.4% identity as determined by pairwise alignment. The h16445 protein shares approximately 36.9% identity over a 130 amino acid overlap with the human IL-2 receptor beta chain, approximately 32.7% identity over a 110 amino acid overlap with the murine IL-2
10 receptor beta chain, approximately 29.7% identity over a 158 amino acid overlap with the human IL-9 receptor, and approximately 28.3% identity over a 166 amino acid overlap with the murine IL-9 receptor, as determined by FASTA.

Figure 2 shows expression of h16445 in various tissues and cell types relative to expression in human hepatoma cell line Hep3B.

15 Figure 3A and 3B shows the effect of the ectopic expression of h16445 on cytokine-induced CAT expression in cells transfected with Type I cytokine receptors.

Figure 4 shows the increase in IL-9 induced CAT expression mediated by an IL-9R/h16445 cytoplasmic-domain receptor chimera.

Figure 5 shows FACs analysis screening of hybridoma supernatants (clone #s 4, 7, or 8) for binding to: (A) the GPI-linked h16445 extracellular domain expressed by
20 HEK293 cells transiently transfected with a plasmid encoding amino acids 1-234 of the extracellular domain of h16445 plus a His tag and the C-terminal signal sequence from human placental alkaline phosphatase (GPI-linker signal), or (B) the h16445 extracellular domain expressed by HEK293 cells transiently transfected with a plasmid encoding the
25 full-length h16445 (B, C, and D). Relative fluorescence intensity exhibited by these transfected cells tagged with particular hybridoma supernatants (represented by peak 2 in panels A-D) is shown versus that exhibited by untransfected cells (represented by peak 1 in panels A-D), which served as the control.

Figure 6 shows FACs analysis screening of h16445-specific hybridoma
30 supernatants (clone #s 3, 4, or 27) for binding to human tonsil CD19+ cells. Expression of h16445 as detected by staining with the h16445-specific hybridoma supernatants (peak

2) is shown relative to that detected by staining with an irrelevant antibody supernatant specific for the chemokine neurotactin (Nt) (peak 1).

DETAILED DESCRIPTION OF THE INVENTION

5

The present invention provides isolated nucleic acid molecules comprising nucleotide sequences encoding the IL-9/IL-2 receptor-like polypeptides whose amino acid sequences are given in SEQ ID NO:2 and SEQ ID NO:4, or a variant or fragment of the polypeptides. Nucleotide sequences encoding the IL-9/IL-2 receptor-like polypeptides of the invention are set forth in SEQ ID NO:1 and SEQ ID NO:3.

10

The disclosed invention relates to methods and compositions for the modulation, diagnosis, and treatment of immune, inflammatory, and respiratory disorders, and disorders associated with the lungs, colon, kidney, and lymphoid tissues, including tonsil and thymus. Immune disorders include, but are not limited to, chronic inflammatory diseases and disorders, such as Crohn's disease, rheumatoid arthritis, reactive arthritis, including Lyme disease, insulin-dependent diabetes, organ-specific autoimmunity, including multiple sclerosis, Hashimoto's thyroiditis and Grave's disease, contact dermatitis, psoriasis, graft rejection, graft versus host disease, sarcoidosis, atopic conditions, such as asthma and allergy, including allergic rhinitis, gastrointestinal allergies, including food allergies, eosinophilia, conjunctivitis, glomerular nephritis, certain pathogen susceptibilities such as helminthic (e.g., leishmaniasis), certain viral infections, including HIV, and bacterial infections, including tuberculosis and lepromatous leprosy.

15

20

Respiratory disorders include, but are not limited to, apnea, asthma, particularly bronchial asthma, berillium disease, bronchiectasis, bronchitis, bronchopneumonia, cystic fibrosis, diphtheria, dyspnea, emphysema, chronic obstructive pulmonary disease, allergic bronchopulmonary aspergillosis, pneumonia, acute pulmonary edema, pertussis, pharyngitis, atelectasis, Wegener's granulomatosis, Legionnaires disease, pleurisy, rheumatic fever, and sinusitis.

25

30

Disorders involving the lung include, but are not limited to, congenital anomalies; atelectasis; diseases of vascular origin, such as pulmonary congestion and edema,

including hemodynamic pulmonary edema and edema caused by microvascular injury, adult respiratory distress syndrome (diffuse alveolar damage), pulmonary embolism, hemorrhage, and infarction, and pulmonary hypertension and vascular sclerosis; chronic obstructive pulmonary disease, such as emphysema, chronic bronchitis, bronchial asthma, and bronchiectasis; diffuse interstitial (infiltrative, restrictive) diseases, such as pneumoconioses, sarcoidosis, idiopathic pulmonary fibrosis, desquamative interstitial pneumonitis, hypersensitivity pneumonitis, pulmonary eosinophilia (pulmonary infiltration with eosinophilia), *Bronchiolitis obliterans*-organizing pneumonia, diffuse pulmonary hemorrhage syndromes, including Goodpasture syndrome, idiopathic pulmonary hemosiderosis and other hemorrhagic syndromes, pulmonary involvement in collagen vascular disorders, and pulmonary alveolar proteinosis; complications of therapies, such as drug-induced lung disease, radiation-induced lung disease, and lung transplantation; tumors, such as bronchogenic carcinoma, including paraneoplastic syndromes, bronchioloalveolar carcinoma, neuroendocrine tumors, such as bronchial carcinoid, miscellaneous tumors, and metastatic tumors; pathologies of the pleura, including inflammatory pleural effusions, noninflammatory pleural effusions, pneumothorax, and pleural tumors, including solitary fibrous tumors (pleural fibroma) and malignant mesothelioma.

Disorders involving the colon include, but are not limited to, congenital anomalies, such as atresia and stenosis, Meckel diverticulum, congenital aganglionic megacolon-Hirschsprung disease; enterocolitis, such as diarrhea and dysentery, infectious enterocolitis, including viral gastroenteritis, bacterial enterocolitis, necrotizing enterocolitis, antibiotic-associated colitis (pseudomembranous colitis), and collagenous and lymphocytic colitis, miscellaneous intestinal inflammatory disorders, including parasites and protozoa, acquired immunodeficiency syndrome, transplantation, drug-induced intestinal injury, radiation enterocolitis, neutropenic colitis (typhlitis), and diversion colitis; idiopathic inflammatory bowel disease, such as Crohn disease and ulcerative colitis; tumors of the colon, such as non-neoplastic polyps, adenomas, familial syndromes, colorectal carcinogenesis, colorectal carcinoma, and carcinoid tumors.

Disorders involving the kidney include, but are not limited to, congenital anomalies including, but not limited to, cystic diseases of the kidney, that include but are not limited to,

cystic renal dysplasia, autosomal dominant (adult) polycystic kidney disease, autosomal recessive (childhood) polycystic kidney disease, and cystic diseases of renal medulla, which include, but are not limited to, medullary sponge kidney, and nephronophthisis-uremic medullary cystic disease complex, acquired (dialysis-associated) cystic disease, such as

5 simple cysts; glomerular diseases including pathologies of glomerular injury that include, but are not limited to, in situ immune complex deposition, that includes, but is not limited to, anti-GBM nephritis, Heymann nephritis, and antibodies against planted antigens, circulating immune complex nephritis, antibodies to glomerular cells, cell-mediated immunity in glomerulonephritis, activation of alternative complement pathway, epithelial cell injury, and

10 pathologies involving mediators of glomerular injury including cellular and soluble mediators, acute glomerulonephritis, such as acute proliferative (poststreptococcal, postinfectious) glomerulonephritis, including but not limited to, poststreptococcal glomerulonephritis and nonstreptococcal acute glomerulonephritis, rapidly progressive (crescentic) glomerulonephritis, nephrotic syndrome, membranous glomerulonephritis

15 (membranous nephropathy), minimal change disease (lipoid nephrosis), focal segmental glomerulosclerosis, membranoproliferative glomerulonephritis, IgA nephropathy (Berger disease), focal proliferative and necrotizing glomerulonephritis (focal glomerulonephritis), hereditary nephritis, including but not limited to, Alport syndrome and thin membrane disease (benign familial hematuria), chronic glomerulonephritis, glomerular lesions

20 associated with systemic disease, including but not limited to, systemic lupus erythematosus, Henoch-Schönlein purpura, bacterial endocarditis, diabetic glomerulosclerosis, amyloidosis, fibrillary and immunotactoid glomerulonephritis, and other systemic disorders; diseases affecting tubules and interstitium, including acute tubular necrosis and tubulointerstitial nephritis, including but not limited to, pyelonephritis and

25 urinary tract infection, acute pyelonephritis, chronic pyelonephritis and reflux nephropathy, and tubulointerstitial nephritis induced by drugs and toxins, including but not limited to, acute drug-induced interstitial nephritis, analgesic abuse nephropathy, nephropathy associated with nonsteroidal anti-inflammatory drugs, and other tubulointerstitial diseases including, but not limited to, urate nephropathy, hypercalcemia and nephrocalcinosis, and

30 multiple myeloma; diseases of blood vessels including benign nephrosclerosis, malignant hypertension and accelerated nephrosclerosis, renal artery stenosis, and thrombotic

microangiopathies including, but not limited to, classic (childhood) hemolytic-uremic syndrome, adult hemolytic-uremic syndrome/thrombotic thrombocytopenic purpura, idiopathic HUS/TTP, and other vascular disorders including, but not limited to, atherosclerotic ischemic renal disease, atheroembolic renal disease, sickle cell disease nephropathy, diffuse cortical necrosis, and renal infarcts; urinary tract obstruction (obstructive uropathy); urolithiasis (renal calculi, stones); and tumors of the kidney including, but not limited to, benign tumors, such as renal papillary adenoma, renal fibroma or hamartoma (renomedullary interstitial cell tumor), angiomyolipoma, and oncocytoma, and malignant tumors, including renal cell carcinoma (hypernephroma, adenocarcinoma of kidney), which includes urothelial carcinomas of renal pelvis.

Disorders involving the thymus include developmental disorders, such as DiGeorge syndrome with thymic hypoplasia or aplasia; thymic cysts; thymic hypoplasia, which involves the appearance of lymphoid follicles within the thymus, creating thymic follicular hyperplasia; and thymomas, including germ cell tumors, lymphomas, Hodgkin disease, and carcinoids. Thymomas can include benign or encapsulated thymoma, and malignant thymoma Type I (invasive thymoma) or Type II, designated thymic carcinoma.

The invention is also directed to methods and compositions for the modulation, diagnosis, and treatment of disorders associated with lymphoid cells including B- and T-cells. These disorders include, but are not limited to, the leukemias, including B-lymphoid leukemias, T-lymphoid leukemias, undifferentiated leukemias; precursor B-cell neoplasms, such as lymphoblastic leukemia/lymphoma; peripheral B-cell neoplasms, including, but not limited to, chronic lymphocytic leukemia/small lymphocytic lymphoma, follicular lymphoma, diffuse large B-cell lymphoma, Burkitt lymphoma, plasma cell neoplasms, multiple myeloma, and related entities, lymphoplasmacytic lymphoma (Waldenström macroglobulinemia), mantle cell lymphoma, marginal zone lymphoma (MALToMa), and hairy cell leukemia.

A novel human full-length cDNA, termed clone h16445, and its murine orthologue, termed clone m16445, are provided. Such sequences are referred to as "IL-9/IL-2 receptor-like" indicating that they share sequence similarity to the IL-9 and IL-2 receptor genes.

The sequences of the invention find use in modulating an immune response. By "modulating" is intended the upregulating or downregulating of a response. That is, the compositions of the invention, affect the targeted activity in either a positive or negative fashion. The activation of T cells is manifested by lymphokine production, cellular proliferation, signaling events, and other effector functions.

The function of T cells is defined by the type of cytokines released upon antigenic challenge. Such cytokines are central to disease evolution in animal models of autoimmunity and infection. Proteins and/or antibodies of the invention are also useful in modulating immune, inflammatory and respiratory responses.

The IL-9/IL-2 receptor-like cDNA, clone h16445, and its murine orthologue, m16445, were identified in a human peripheral blood lymphocyte cDNA library and a mouse LTBMCL (long-term bone marrow cell) cDNA library, respectively. Clone h16445 encodes an approximately 2.3 Kb mRNA transcript having the corresponding cDNA set forth in SEQ ID NO:1. This transcript has a 1614 nucleotide open reading frame (nucleotides 349-1965 of SEQ ID NO:1), which encodes a 538 amino acid protein (SEQ ID NO:2) having a molecular weight of approximately 59.1 kDa. An analysis of the full-length h16445 polypeptide predicts that the N-terminal 19 amino acids represent a signal peptide. Transmembrane segments from amino acids (aa) 9-26 and 425-446 were predicted by MEMSAT. Transmembrane segments were also predicted from aa 219-236 and 406-427 of the presumed mature peptide sequence. Prosite program analysis was used to predict various sites within the h16445 protein. N-glycosylation sites were predicted at aa 73-76, 97-100, 104-107, 125-128, and 135-138. A cAMP- and cGMP-dependent protein kinase phosphorylation site was predicted at aa 191-194. Protein kinase C phosphorylation sites were predicted at aa 117-119, 137-139, 170-172, and 254-256. Casein kinase II phosphorylation sites were predicted at aa 50-53, 140-143, 194-197, 213-216, 225-228, 299-302, 312-315, 380-383, 393-396, 444-447, 474-477, 501-504, and 509-512. A tyrosine kinase phosphorylation site was predicted at aa 153-160. N-myristoylation sites were predicted at aa 293-298, 360-365, 429-434, 439-444, 462-467, 472-477, 479-484, and 496-501.

An RGD cell attachment sequence was predicted at aa 163-165. A growth factor and cytokine receptor signature 2 sequence was predicted at aa 212-218. The IL-9/IL-2

receptor-like protein possesses a fibronectin type III domain, from aa 120-215, and a U-
PAR/Ly-6 domain, from aa 230-255, as predicted by HMMer, Version 2. The fibronectin
type III domain is one of three types of internal repeats within the plasma protein
fibronectin. The tenth fibronectin type III repeat contains an RGD cell recognition
5 sequence in a flexible loop between two strands. Type III modules are present in both
extracellular and intracellular proteins. See, for example, Petersen *et al.* (1983) *Proc.*
Natl. Acad. Sci. USA 80:137-141. The U-PAR/Ly-6 domain is a urokinase plasminogen
activator surface receptor involved in binding urokinase plasminogen activator. This
domain is responsible for signal transduction and is found in the family of Ly-6 T-cell
10 antigens. See, for example, Behrendt *et al.* (1991) *J. Biol. Chem.* 266:7842-7847, and
Ploug *et al.* (1993) *J. Biol. Chem.* 268:17539-17546.

Sub
A2
The h16445 protein displays similarity to the human IL-2 receptor beta chain
(SEQ ID NO:5; approximately 36.9% identity over a 130 amino acid overlap), the murine
IL-2 receptor beta chain (SEQ ID NO:6; 32.7% identity over a 110 amino acid overlap),
15 the human IL-9 receptor (SEQ ID NO:7; approximately 29.7% identity over a 158 amino
acid overlap), and the murine IL-9 receptor (SEQ ID NO:8; approximately 28.3% identity
over a 166 amino acid overlap) (see Figure 1).

A plasmid containing the h16445 cDNA insert was deposited with American
Type Culture Collection (ATCC), 10801 University Boulevard, Manassas, Virginia, on
20 _____, and assigned Accession Number _____. This deposit will be
maintained under the terms of the Budapest Treaty on the International Recognition of
the Deposit of Microorganisms for the Purposes of Patent Procedure. This deposit was
made merely as a convenience for those of skill in the art and is not an admission that a
deposit is required under 35 U.S.C. § 112.

Sub
A3
25 The murine clone, m16445, encodes an approximately 2.5 Kb mRNA transcript
having the corresponding cDNA set forth in SEQ ID NO:3. This transcript has a 1587
nucleotide open reading frame (nucleotides 391-1976 of SEQ ID NO:3), which encodes a
529 amino acid protein (SEQ ID NO:4) having a molecular weight of approximately 58.3
kDa. An analysis of the full-length m16445 polypeptide predicts that the N-terminal 19
30 amino acids represent a signal peptide. This polypeptide represents the protein sequence
encoded by the murine orthologue of the h16445 gene. The mouse 16445 protein shares

Sub
Q3
cont

approximately 64.4% identity with the human ~~16445~~ protein disclosed in SEQ ID NO:2 (see Figure 1).

An analysis of the disclosed m16445 polypeptide sequence (SEQ ID NO:4) using the MEMSAT program predicts transmembrane segments from amino acids (aa) 7-23 and 415-434. Transmembrane segments were also predicted from aa 219-235 and 396-415 of the presumed mature peptide sequence. Prosite program analysis was also used to predict various sites within the m16445 protein sequence. N-glycosylation sites were predicted at 73-76, 97-100, 104-107, 125-128, and 182-185. A glycosaminoglycan attachment site was predicted from aa 430-433. Protein kinase C phosphorylation sites were predicted at aa 117-119, 131-133, and 209-211. Casein kinase II phosphorylation sites were predicted at aa 19-22, 50-53, 140-143, 213-216, 299-302, 378-381, 391-394, 442-445, 472-475, and 498-501. A tyrosine kinase phosphorylation site was predicted at 153-160. N-myristoylation sites were predicted at 16-21, 355-360, 427-432, 433-438, 466-471, 477-482, and 493-498. A growth factor and cytokine receptor signature 2 sequence was predicted at aa 212-218. Analysis with HMMer, Version 2, predicted a fibronectin type III domain from aa 120-215 in the mouse IL-9/IL-2 receptor-like protein, similar to that described for the human 16445 protein. A FN3_2 domain from aa 120-209 was also predicted for this protein by HMMer analysis.

The IL-9/IL-2 receptor-like sequences of the invention are members of a family of molecules (the "Type 1 cytokine receptor family") having conserved functional features. The term "family" when referring to the proteins and nucleic acid molecules of the invention is intended to mean two or more proteins or nucleic acid molecules having sufficient amino acid or nucleotide sequence identity as defined herein. Such family members can be naturally occurring and can be from either the same or different species. For example, a family can contain a first protein of murine origin and a homologue of that protein of human origin, as well as a second, distinct protein of human origin and a murine homologue of that protein. Members of a family may also have common functional characteristics.

Preferred IL-9/IL-2 receptor-like polypeptides of the present invention have an amino acid sequence sufficiently identical to the amino acid sequence of SEQ ID NO:2 or 4. The term "sufficiently identical" is used herein to refer to a first amino acid or

nucleotide sequence that contains a sufficient or minimum number of identical or equivalent (e.g., with a similar side chain) amino acid residues or nucleotides to a second amino acid or nucleotide sequence such that the first and second amino acid or nucleotide sequences have a common structural domain and/or common functional activity. For example, amino acid or nucleotide sequences that contain a common structural domain having at least about 45%, 55%, or 65% identity, preferably 70% identity, more preferably 80%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97% or 98% identity are defined herein as sufficiently identical.

To determine the percent identity of two amino acid sequences or of two nucleic acids, the sequences are aligned for optimal comparison purposes. The percent identity between the two sequences is a function of the number of identical positions shared by the sequences (i.e., percent identity = number of identical positions/total number of positions (e.g., overlapping positions) x 100). In one embodiment, the two sequences are the same length. The percent identity between two sequences can be determined using techniques similar to those described below, with or without allowing gaps. In calculating percent identity, only exact matches are counted.

The determination of percent identity between two sequences can be accomplished using a mathematical algorithm. A preferred, nonlimiting example of a mathematical algorithm utilized for the comparison of two sequences is the algorithm of Karlin and Altschul (1990) *Proc. Natl. Acad. Sci. USA* 87:2264, modified as in Karlin and Altschul (1993) *Proc. Natl. Acad. Sci. USA* 90:5873-5877. Such an algorithm is incorporated into the NBLAST and XBLAST programs of Altschul *et al.* (1990) *J. Mol. Biol.* 215:403. BLAST nucleotide searches can be performed with the NBLAST program, score = 100, wordlength = 12, to obtain nucleotide sequences homologous to IL-9/IL-2 receptor-like nucleic acid molecules of the invention. BLAST protein searches can be performed with the XBLAST program, score = 50, wordlength = 3, to obtain amino acid sequences homologous to IL-9/IL-2 receptor-like protein molecules of the invention. To obtain gapped alignments for comparison purposes, Gapped BLAST can be utilized as described in Altschul *et al.* (1997) *Nucleic Acids Res.* 25:3389. Alternatively, PSI-Blast can be used to perform an iterated search that detects distant relationships between molecules. See Altschul *et al.* (1997) *supra*. When utilizing

BLAST, Gapped BLAST, and PSI-Blast programs, the default parameters of the respective programs (e.g., XBLAST and NBLAST) can be used. *See* <http://www.ncbi.nlm.nih.gov>. Another preferred, non-limiting example of a mathematical algorithm utilized for the comparison of sequences is the algorithm of Myers and Miller (1988) *CABIOS* 4:11-17. Such an algorithm is incorporated into the ALIGN program (version 2.0), which is part of the GCG sequence alignment software package. When utilizing the ALIGN program for comparing amino acid sequences, a PAM120 weight residue table, a gap length penalty of 12, and a gap penalty of 4 can be used.

10 Accordingly, another embodiment of the invention features isolated IL-9/IL-2 receptor-like proteins and polypeptides having an IL-9/IL-2 receptor-like protein activity. As used interchangeably herein, a "IL-9/IL-2 receptor-like protein activity", "biological activity of an IL-9/IL-2 receptor-like protein", or "functional activity of an IL-9/IL-2 receptor-like protein" refers to an activity exerted by an IL-9/IL-2 receptor-like protein, polypeptide, or nucleic acid molecule on an IL-9/IL-2 receptor-like responsive cell as determined *in vivo*, or *in vitro*, according to standard assay techniques. An IL-9/IL-2 receptor-like activity can be a direct activity, such as an association with or an enzymatic activity on a second protein, or an indirect activity, such as a cellular signaling activity mediated by interaction of the IL-9/IL-2 receptor-like protein with a second protein. In a preferred embodiment, an IL-9/IL-2 receptor-like activity includes at least one or more of the following activities: (1) modulating (stimulating and/or enhancing or inhibiting) cellular proliferation, differentiation, and/or function, particularly immune cells, for example lymphocytes, such as B cells, plasma cells, T cells, and null cells, macrophages, histiocytes, and granulocytes, such as neutrophils, eosinophils, basophils, and tissue mast cells; (2) modulating an IL-9/IL-2 receptor-like immune response; (3) modulating an inflammatory response; (4) modulating a respiratory response; and (5) binding an IL-9 or IL-2 receptor ligand.

 An "isolated" or "purified" IL-9/IL-2 receptor-like nucleic acid molecule or protein, or biologically active portion thereof, is substantially free of other cellular material, or culture medium when produced by recombinant techniques, or substantially free of chemical precursors or other chemicals when chemically synthesized. Preferably,

an "isolated" nucleic acid is free of sequences (preferably protein encoding sequences) that naturally flank the nucleic acid (i.e., sequences located at the 5' and 3' ends of the nucleic acid) in the genomic DNA of the organism from which the nucleic acid is derived. For purposes of the invention, "isolated" when used to refer to nucleic acid molecules excludes isolated chromosomes. For example, in various embodiments, the isolated IL-9/IL-2 receptor-like nucleic acid molecule can contain less than about 5 kb, 4 kb, 3 kb, 2 kb, 1 kb, 0.5 kb, or 0.1 kb of nucleotide sequences that naturally flank the nucleic acid molecule in genomic DNA of the cell from which the nucleic acid is derived. An IL-9/IL-2 receptor-like protein that is substantially free of cellular material includes preparations of IL-9/IL-2 receptor-like protein having less than about 30%, 20%, 10%, or 5% (by dry weight) of non-IL-9/IL-2 receptor-like protein (also referred to herein as a "contaminating protein"). When the IL-9/IL-2 receptor-like protein or biologically active portion thereof is recombinantly produced, preferably, culture medium represents less than about 30%, 20%, 10%, or 5% of the volume of the protein preparation. When IL-9/IL-2 receptor-like protein is produced by chemical synthesis, preferably the protein preparations have less than about 30%, 20%, 10%, or 5% (by dry weight) of chemical precursors or non-IL-9/IL-2 receptor-like chemicals.

Various aspects of the invention are described in further detail in the following subsections.

I. Isolated Nucleic Acid Molecules

One aspect of the invention pertains to isolated nucleic acid molecules comprising nucleotide sequences encoding IL-9/IL-2 receptor-like proteins and polypeptides or biologically active portions thereof, as well as nucleic acid molecules sufficient for use as hybridization probes to identify IL-9/IL-2 receptor-like-encoding nucleic acids (e.g., IL-9/IL-2 receptor-like mRNA) and fragments for use as PCR primers for the amplification or mutation of IL-9/IL-2 receptor-like nucleic acid molecules. As used herein, the term "nucleic acid molecule" is intended to include DNA molecules (e.g., cDNA or genomic DNA) and RNA molecules (e.g., mRNA) and analogs of the DNA or RNA generated using nucleotide analogs. The nucleic acid molecule can be single-stranded or double-stranded, but preferably is double-stranded DNA.

Nucleotide sequences encoding the IL-9/IL-2 receptor-like proteins of the present invention include sequences set forth in SEQ ID NO:1 and SEQ ID NO:3, the nucleotide sequence of the cDNA insert of the plasmid deposited with the ATCC as Accession Number _____ (the "cDNA of ATCC _____"), and complements thereof. By

5 "complement" is intended a nucleotide sequence that is sufficiently complementary to a given nucleotide sequence such that it can hybridize to the given nucleotide sequence to thereby form a stable duplex. The corresponding amino acid sequences for the IL-9/IL-2 receptor-like proteins encoded by these nucleotide sequences are set forth in SEQ ID NO:2 and SEQ ID NO:4.

10 Nucleic acid molecules that are fragments of these IL-9/IL-2 receptor-like nucleotide sequences are also encompassed by the present invention. By "fragment" is intended a portion of the nucleotide sequence encoding an IL-9/IL-2 receptor-like protein. A fragment of an IL-9/IL-2 receptor-like nucleotide sequence may encode a biologically active portion of an IL-9/IL-2 receptor-like protein, or it may be a fragment
15 that can be used as a hybridization probe or PCR primer using methods disclosed below. A biologically active portion of an IL-9/IL-2 receptor-like protein can be prepared by isolating a portion of one of the IL-9/IL-2 receptor-like nucleotide sequences of the invention, expressing the encoded portion of the IL-9/IL-2 receptor-like protein (e.g., by recombinant expression *in vitro*), and assessing the activity of the encoded portion of the
20 IL-9/IL-2 receptor-like protein. Nucleic acid molecules that are fragments of an IL-9/IL-2 receptor-like nucleotide sequence comprise at least 15, 20, 50, 75, 100, 200, 300, 350, 400, 450, 500, 550, 600, 650, 700, 750, 800, 850, 900, 950, 1000, 1050, 1100, 1150, 1200, 1250, 1300, 1350, 1400 nucleotides, or up to the number of nucleotides present in a full-length IL-9/IL-2 receptor-like nucleotide sequence disclosed herein (for example,
25 2343 or 2456 nucleotides for SEQ ID NO:1 or SEQ ID NO:3, respectively) depending upon the intended use.

It is understood that isolated fragments include any contiguous sequence not disclosed prior to the invention as well as sequences that are substantially the same and which are not disclosed. Accordingly, if an isolated fragment is disclosed prior to the
30 present invention, that fragment is not intended to be encompassed by the invention. When a sequence is not disclosed prior to the present invention, an isolated nucleic acid

fragment is at least about 12, 15, 20, 25, or 30 contiguous nucleotides. Other regions of the nucleotide sequence may comprise fragments of various sizes, depending upon potential homology with previously disclosed sequences.

For example, when considering the open reading frame of SEQ ID NO:1 (nt 349-1965), the nucleotide sequence from about 349 to about 398 encompasses isolated fragments greater than about 25, 27, or 30 nucleotides; the nucleotide sequence from about 398 to about 508 encompasses isolated fragments greater than about 106, 107, or 108 nucleotides; the nucleotide sequence from about 508 to about 858 encompasses isolated fragments greater than about 201, 205, or 210 nucleotides; the nucleotide sequence from about 858 to about 1158 encompasses isolated fragments greater than about 179, 185, or 190 nucleotides; the nucleotide sequence from about 1158 to about 1965 encompasses isolated fragments greater than about 752, 755, or 780 nucleotides.

A fragment of an IL-9/IL-2 receptor-like nucleotide sequence that encodes a biologically active portion of an IL-9/IL-2 receptor-like protein of the invention will encode at least 15, 25, 30, 50, 75, 100, 125, 150, 175, 200, 250, or 300 contiguous amino acids, or up to the total number of amino acids present in a full-length IL-9/IL-2 receptor-like protein of the invention (for example, 538 amino acids for SEQ ID NO:2, or 529 amino acids for SEQ ID NO:4). Fragments of an IL-9/IL-2 receptor-like nucleotide sequence that are useful as hybridization probes for PCR primers generally need not encode a biologically active portion of an IL-9/IL-2 receptor-like protein.

Nucleic acid molecules that are variants of the IL-9/IL-2 receptor-like nucleotide sequences disclosed herein are also encompassed by the present invention. "Variants" of the IL-9/IL-2 receptor-like nucleotide sequences include those sequences that encode the IL-9/IL-2 receptor-like proteins disclosed herein but that differ conservatively because of the degeneracy of the genetic code. These naturally occurring allelic variants can be identified with the use of well-known molecular biology techniques, such as polymerase chain reaction (PCR) and hybridization techniques as outlined below. Variant nucleotide sequences also include synthetically derived nucleotide sequences that have been generated, for example, by using site-directed mutagenesis but which still encode the IL-9/IL-2 receptor-like proteins disclosed in the present invention as discussed below. Generally, nucleotide sequence variants of the invention will have at least 45%, 55%,

65%, 75%, 85%, 95%, or 98% identity to a particular nucleotide sequence disclosed herein. A variant IL-9/IL-2 receptor-like nucleotide sequence will encode an IL-9/IL-2 receptor-like protein that has an amino acid sequence having at least 45%, 55%, 65%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97% or 98% identity to the amino acid sequence of an IL-9/IL-2 receptor-like protein disclosed herein.

In addition to the IL-9/IL-2 receptor-like nucleotide sequences shown in SEQ ID NOs: 1 and 3, and the nucleotide sequence of the cDNA of ATCC _____, it will be appreciated by those skilled in the art that DNA sequence polymorphisms that lead to changes in the amino acid sequences of IL-9/IL-2 receptor-like proteins may exist within a population (e.g., the human population). Such genetic polymorphism in an IL-9/IL-2 receptor-like gene may exist among individuals within a population due to natural allelic variation. An allele is one of a group of genes that occur alternatively at a given genetic locus. As used herein, the terms "gene" and "recombinant gene" refer to nucleic acid molecules comprising an open reading frame encoding an IL-9/IL-2 receptor-like protein, preferably a mammalian IL-9/IL-2 receptor-like protein. As used herein, the phrase "allelic variant" refers to a nucleotide sequence that occurs at an IL-9/IL-2 receptor-like locus or to a polypeptide encoded by the nucleotide sequence. Such natural allelic variations can typically result in 1-5% variance in the nucleotide sequence of the IL-9/IL-2 receptor-like gene. Any and all such nucleotide variations and resulting amino acid polymorphisms or variations in an IL-9/IL-2 receptor-like sequence that are the result of natural allelic variation and that do not alter the functional activity of IL-9/IL-2 receptor-like proteins are intended to be within the scope of the invention.

Moreover, nucleic acid molecules encoding IL-9/IL-2 receptor-like proteins from other species (IL-9/IL-2 receptor-like homologues), which have a nucleotide sequence differing from that of the IL-9/IL-2 receptor-like sequences disclosed herein, are intended to be within the scope of the invention. For example, nucleic acid molecules corresponding to natural allelic variants and homologues of the human IL-9/IL-2 receptor-like cDNA of the invention can be isolated based on their identity to the human IL-9/IL-2 receptor-like nucleic acid disclosed herein using the human cDNA, or a portion thereof, as a hybridization probe according to standard hybridization techniques under stringent hybridization conditions as disclosed below.

In addition to naturally-occurring allelic variants of the IL-9/IL-2 receptor-like sequences that may exist in the population, the skilled artisan will further appreciate that changes can be introduced by mutation into the nucleotide sequences of the invention thereby leading to changes in the amino acid sequence of the encoded IL-9/IL-2 receptor-like proteins, without altering the biological activity of the IL-9/IL-2 receptor-like proteins. Thus, an isolated nucleic acid molecule encoding an IL-9/IL-2 receptor-like protein having a sequence that differs from that of SEQ ID NO:2 or 4 can be created by introducing one or more nucleotide substitutions, additions, or deletions into the corresponding nucleotide sequence disclosed herein, such that one or more amino acid substitutions, additions or deletions are introduced into the encoded protein. Mutations can be introduced by standard techniques, such as site-directed mutagenesis and PCR-mediated mutagenesis. Such variant nucleotide sequences are also encompassed by the present invention.

For example, preferably, conservative amino acid substitutions may be made at one or more predicted, preferably nonessential amino acid residues. A "nonessential" amino acid residue is a residue that can be altered from the wild-type sequence of an IL-9/IL-2 receptor-like protein (e.g., the sequence of SEQ ID NO:2 or 4) without altering the biological activity, whereas an "essential" amino acid residue is required for biological activity. A "conservative amino acid substitution" is one in which the amino acid residue is replaced with an amino acid residue having a similar side chain. Families of amino acid residues having similar side chains have been defined in the art. These families include amino acids with basic side chains (e.g., lysine, arginine, histidine), acidic side chains (e.g., aspartic acid, glutamic acid), uncharged polar side chains (e.g., glycine, asparagine, glutamine, serine, threonine, tyrosine, cysteine), nonpolar side chains (e.g., alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, tryptophan), beta-branched side chains (e.g., threonine, valine, isoleucine) and aromatic side chains (e.g., tyrosine, phenylalanine, tryptophan, histidine). Such substitutions would not be made for conserved amino acid residues, or for amino acid residues residing within a conserved motif, such as the growth factor and cytokine receptor signature 2 sequence of SEQ ID NO:2 and 4 and the U-PAR/Ly-6 domain sequence of SEQ ID NO:2, where such residues are essential for protein activity.

Alternatively, variant IL-9/IL-2 receptor-like nucleotide sequences can be made by introducing mutations randomly along all or part of an IL-9/IL-2 receptor-like coding sequence, such as by saturation mutagenesis, and the resultant mutants can be screened for IL-9/IL-2 receptor-like biological activity to identify mutants that retain activity.

- 5 Following mutagenesis, the encoded protein can be expressed recombinantly, and the activity of the protein can be determined using standard assay techniques.

Thus the nucleotide sequences of the invention include the sequences disclosed herein as well as fragments and variants thereof. The IL-9/IL-2 receptor-like nucleotide sequences of the invention, and fragments and variants thereof, can be used as probes
10 and/or primers to identify and/or clone IL-9/IL-2 receptor-like homologues in other cell types, e.g., from other tissues, as well as IL-9/IL-2 receptor-like homologues from other mammals. Such probes can be used to detect transcripts or genomic sequences encoding the same or identical proteins. These probes can be used as part of a diagnostic test kit for identifying cells or tissues that misexpress an IL-9/IL-2 receptor-like protein, such as
15 by measuring levels of an IL-9/IL-2 receptor-like-encoding nucleic acid in a sample of cells from a subject, e.g., detecting IL-9/IL-2 receptor-like mRNA levels or determining whether a genomic IL-9/IL-2 receptor-like gene has been mutated or deleted.

In this manner, methods such as PCR, hybridization, and the like can be used to identify such sequences having substantial identity to the sequences of the invention.
20 See, for example, Sambrook *et al.* (1989) *Molecular Cloning: Laboratory Manual* (2d ed., Cold Spring Harbor Laboratory Press, Plainview, NY) and Innis, *et al.* (1990) *PCR Protocols: A Guide to Methods and Applications* (Academic Press, NY). IL-9/IL-2 receptor-like nucleotide sequences isolated based on their sequence identity to the IL-9/IL-2 receptor-like nucleotide sequences set forth herein or to fragments and variants
25 thereof are encompassed by the present invention.

In a hybridization method, all or part of a known IL-9/IL-2 receptor-like nucleotide sequence can be used to screen cDNA or genomic libraries. Methods for construction of such cDNA and genomic libraries are generally known in the art and are disclosed in Sambrook *et al.* (1989) *Molecular Cloning: A Laboratory Manual* (2d ed.,
30 Cold Spring Harbor Laboratory Press, Plainview, NY). The so-called hybridization probes may be genomic DNA fragments, cDNA fragments, RNA fragments, or other

oligonucleotides, and may be labeled with a detectable group such as ^{32}P , or any other detectable marker, such as other radioisotopes, a fluorescent compound, an enzyme, or an enzyme co-factor. Probes for hybridization can be made by labeling synthetic oligonucleotides based on the known IL-9/IL-2 receptor-like nucleotide sequence disclosed herein. Degenerate primers designed on the basis of conserved nucleotides or amino acid residues in a known IL-9/IL-2 receptor-like nucleotide sequence or encoded amino acid sequence can additionally be used. The probe typically comprises a region of nucleotide sequence that hybridizes under stringent conditions to at least about 12, preferably about 25, more preferably about 50, 75, 100, 125, 150, 175, 200, 250, 300, 350, or 400 consecutive nucleotides of an IL-9/IL-2 receptor-like nucleotide sequence of the invention or a fragment or variant thereof. Preparation of probes for hybridization is generally known in the art and is disclosed in Sambrook *et al.* (1989) *Molecular Cloning: A Laboratory Manual* (2d ed., Cold Spring Harbor Laboratory Press, Plainview, New York), herein incorporated by reference.

For example, in one embodiment, a previously unidentified IL-9/IL-2 receptor-like nucleic acid molecule hybridizes under stringent conditions to a probe that is a nucleic acid molecule comprising one of the IL-9/IL-2 receptor-like nucleotide sequences of the invention or a fragment thereof. In another embodiment, the previously unknown IL-9/IL-2 receptor-like nucleic acid molecule is at least 300, 325, 350, 375, 400, 425, 450, 500, 550, 600, 650, 700, 800, 900, 1000, 2,000, 3,000, 4,000 or 5,000 nucleotides in length and hybridizes under stringent conditions to a probe that is a nucleic acid molecule comprising one of the IL-9/IL-2 receptor-like nucleotide sequences disclosed herein or a fragment thereof.

Accordingly, in another embodiment, an isolated previously unknown IL-9/IL-2 receptor-like nucleic acid molecule of the invention is at least 300, 325, 350, 375, 400, 425, 450, 500, 550, 600, 650, 700, 800, 900, 1000, 1,100, 1,200, 1,300, or 1,400 nucleotides in length and hybridizes under stringent conditions to a probe that is a nucleic acid molecule comprising one of the nucleotide sequences of the invention, preferably the coding sequence set forth in SEQ ID NO:1 or 3, the cDNA of ATCC _____, or a complement, fragment, or variant thereof.

As used herein, the term "hybridizes under stringent conditions" is intended to describe conditions for hybridization and washing under which nucleotide sequences having at least 60%, 65%, 70%, preferably 75% identity to each other typically remain hybridized to each other. Such stringent conditions are known to those skilled in the art and can be found in *Current Protocols in Molecular Biology* (John Wiley & Sons, New York (1989)), 6.3.1-6.3.6. A preferred, non-limiting example of stringent hybridization conditions is hybridization in 6X sodium chloride/sodium citrate (SSC) at about 45°C, followed by one or more washes in 0.2 X SSC, 0.1% SDS at 50-65°C. In another preferred embodiment, stringent conditions comprise hybridization in 6 X SSC at 42°C, followed by washing with 1 X SSC at 55°C. Preferably, an isolated nucleic acid molecule that hybridizes under stringent conditions to an IL-9/IL-2 receptor-like sequence of the invention corresponds to a naturally-occurring nucleic acid molecule. As used herein, a "naturally-occurring" nucleic acid molecule refers to an RNA or DNA molecule having a nucleotide sequence that occurs in nature (e.g., encodes a natural protein).

Thus, in addition to the IL-9/IL-2 receptor-like nucleotide sequences disclosed herein and fragments and variants thereof, the isolated nucleic acid molecules of the invention also encompass homologous DNA sequences identified and isolated from other cells and/or organisms by hybridization with entire or partial sequences obtained from the IL-9/IL-2 receptor-like nucleotide sequences disclosed herein or variants and fragments thereof.

The present invention also encompasses antisense nucleic acid molecules, i.e., molecules that are complementary to a sense nucleic acid encoding a protein, e.g., complementary to the coding strand of a double-stranded cDNA molecule, or complementary to an mRNA sequence. Accordingly, an antisense nucleic acid can hydrogen bond to a sense nucleic acid. The antisense nucleic acid can be complementary to an entire IL-9/IL-2 receptor-like coding strand, or to only a portion thereof, e.g., all or part of the protein coding region (or open reading frame). An antisense nucleic acid molecule can be antisense to a noncoding region of the coding strand of a nucleotide sequence encoding an IL-9/IL-2 receptor-like protein. The noncoding regions are the 5' and 3' sequences that flank the coding region and are not translated into amino acids.

Given the coding-strand sequence encoding an IL-9/IL-2 receptor-like protein disclosed herein (e.g., SEQ ID NO:1 or 3), antisense nucleic acids of the invention can be designed according to the rules of Watson and Crick base pairing. The antisense nucleic acid molecule can be complementary to the entire coding region of IL-9/IL-2 receptor-like mRNA, but more preferably is an oligonucleotide that is antisense to only a portion of the coding or noncoding region of IL-9/IL-2 receptor-like mRNA. For example, the antisense oligonucleotide can be complementary to the region surrounding the translation start site of IL-9/IL-2 receptor-like mRNA. An antisense oligonucleotide can be, for example, about 5, 10, 15, 20, 25, 30, 35, 40, 45, or 50 nucleotides in length. An antisense nucleic acid of the invention can be constructed using chemical synthesis and enzymatic ligation procedures known in the art.

For example, an antisense nucleic acid (e.g., an antisense oligonucleotide) can be chemically synthesized using naturally occurring nucleotides or variously modified nucleotides designed to increase the biological stability of the molecules or to increase the physical stability of the duplex formed between the antisense and sense nucleic acids, including, but not limited to, for example e.g., phosphorothioate derivatives and acridine substituted nucleotides. Alternatively, the antisense nucleic acid can be produced biologically using an expression vector into which a nucleic acid has been subcloned in an antisense orientation (i.e., RNA transcribed from the inserted nucleic acid will be of an antisense orientation to a target nucleic acid of interest, described further in the following subsection).

The antisense nucleic acid molecules of the invention are typically administered to a subject or generated *in situ* such that they hybridize with or bind to cellular mRNA and/or genomic DNA encoding an IL-9/IL-2 receptor-like protein to thereby inhibit expression of the protein, e.g., by inhibiting transcription and/or translation. An example of a route of administration of antisense nucleic acid molecules of the invention includes direct injection at a tissue site. Alternatively, antisense nucleic acid molecules can be modified to target selected cells and then administered systemically. For example, antisense molecules can be linked to peptides or antibodies to form a complex that specifically binds to receptors or antigens expressed on a selected cell surface. The antisense nucleic acid molecules can also be delivered to cells using the vectors described

herein. To achieve sufficient intracellular concentrations of the antisense molecules, vector constructs in which the antisense nucleic acid molecule is placed under the control of a strong pol II or pol III promoter are preferred.

An antisense nucleic acid molecule of the invention can be an α -anomeric nucleic acid molecule. An α -anomeric nucleic acid molecule forms specific double-stranded hybrids with complementary RNA in which, contrary to the usual β -units, the strands run parallel to each other (Gaultier *et al.* (1987) *Nucleic Acids Res.* 15:6625-6641). The antisense nucleic acid molecule can also comprise a 2'-o-methylribonucleotide (Inoue *et al.* (1987) *Nucleic Acids Res.* 15:6131-6148) or a chimeric RNA-DNA analogue (Inoue *et al.* (1987) *FEBS Lett.* 215:327-330).

The invention also encompasses ribozymes, which are catalytic RNA molecules with ribonuclease activity that are capable of cleaving a single-stranded nucleic acid, such as an mRNA, to which they have a complementary region. Ribozymes (e.g., hammerhead ribozymes (described in Haselhoff and Gerlach (1988) *Nature* 334:585-591)) can be used to catalytically cleave IL-9/IL-2 receptor-like mRNA transcripts to thereby inhibit translation of IL-9/IL-2 receptor-like mRNA. A ribozyme having specificity for an IL-9/IL-2 receptor-like-encoding nucleic acid can be designed based upon the nucleotide sequence of an IL-9/IL-2 receptor-like cDNA disclosed herein (e.g., SEQ ID NO:1 or 3). *See, e.g.,* Cech *et al.*, U.S. Patent No. 4,987,071; and Cech *et al.*, U.S. Patent No. 5,116,742. Alternatively, IL-9/IL-2 receptor-like mRNA can be used to select a catalytic RNA having a specific ribonuclease activity from a pool of RNA molecules. *See, e.g.,* Bartel and Szostak (1993) *Science* 261:1411-1418.

The invention also encompasses nucleic acid molecules that form triple helical structures. For example, IL-9/IL-2 receptor-like gene expression can be inhibited by targeting nucleotide sequences complementary to the regulatory region of the IL-9/IL-2 receptor-like protein (e.g., the IL-9/IL-2 receptor-like promoter and/or enhancers) to form triple helical structures that prevent transcription of the IL-9/IL-2 receptor-like gene in target cells. *See generally* Helene (1991) *Anticancer Drug Des.* 6(6):569; Helene (1992) *Ann. N.Y. Acad. Sci.* 660:27; and Maher (1992) *Bioassays* 14(12):807.

In preferred embodiments, the nucleic acid molecules of the invention can be modified at the base moiety, sugar moiety, or phosphate backbone to improve, e.g., the

stability, hybridization, or solubility of the molecule. For example, the deoxyribose phosphate backbone of the nucleic acids can be modified to generate peptide nucleic acids (see Hyrup *et al.* (1996) *Bioorganic & Medicinal Chemistry* 4:5). As used herein, the terms "peptide nucleic acids" or "PNAs" refer to nucleic acid mimics, e.g., DNA mimics, in which the deoxyribose phosphate backbone is replaced by a pseudopeptide backbone and only the four natural nucleobases are retained. The neutral backbone of PNAs has been shown to allow for specific hybridization to DNA and RNA under conditions of low ionic strength. The synthesis of PNA oligomers can be performed using standard solid-phase peptide synthesis protocols as described in Hyrup *et al.* (1996), *supra*; Perry-O'Keefe *et al.* (1996) *Proc. Natl. Acad. Sci. USA* 93:14670.

PNAs of an IL-9/IL-2 receptor-like molecule can be used in therapeutic and diagnostic applications. For example, PNAs can be used as antisense or antigene agents for sequence-specific modulation of gene expression by, e.g., inducing transcription or translation arrest or inhibiting replication. PNAs of the invention can also be used, e.g., in the analysis of single base pair mutations in a gene by, e.g., PNA-directed PCR clamping; as artificial restriction enzymes when used in combination with other enzymes, e.g., S1 nucleases (Hyrup (1996), *supra*; or as probes or primers for DNA sequence and hybridization (Hyrup (1996), *supra*; Perry-O'Keefe *et al.* (1996), *supra*).

In another embodiment, PNAs of an IL-9/IL-2 receptor-like molecule can be modified, e.g., to enhance their stability, specificity, or cellular uptake, by attaching lipophilic or other helper groups to PNA, by the formation of PNA-DNA chimeras, or by the use of liposomes or other techniques of drug delivery known in the art. The synthesis of PNA-DNA chimeras can be performed as described in Hyrup (1996), *supra*; Finn *et al.* (1996) *Nucleic Acids Res.* 24(17):3357-63; Mag *et al.* (1989) *Nucleic Acids Res.* 17:5973; and Peterson *et al.* (1975) *Bioorganic Med. Chem. Lett.* 5:1119.

II. Isolated IL-9/IL-2 Receptor-like Proteins and Anti-IL-9/IL-2 Receptor-like Antibodies

IL-9/IL-2 receptor-like proteins are also encompassed within the present invention. By "IL-9/IL-2 receptor-like protein" is intended a protein having the amino

acid sequence set forth in SEQ ID NO: 2 or SEQ ID NO:4, as well as fragments, biologically active portions, and variants thereof.

"Fragments" or "biologically active portions" include polypeptide fragments suitable for use as immunogens to raise anti-IL-9/IL-2 receptor-like antibodies.

- 5 Fragments include peptides comprising amino acid sequences sufficiently identical to or derived from the amino acid sequence of an IL-9/IL-2 receptor-like protein of the invention and exhibiting at least one activity of an IL-9/IL-2 receptor-like protein, but which include fewer amino acids than the full-length SEQ ID NO:2 or SEQ ID NO:4 IL-9/IL-2 receptor-like proteins disclosed herein. Typically, biologically active portions
- 10 comprise a domain or motif with at least one activity of the IL-9/IL-2 receptor-like protein. A biologically active portion of an IL-9/IL-2 receptor-like protein can be a polypeptide which is, for example, 10, 25, 50, 100 or more amino acids in length. Such biologically active portions can be prepared by recombinant techniques and evaluated for one or more of the functional activities of a native IL-9/IL-2 receptor-like protein. As
- 15 used here, a fragment comprises at least 5 contiguous amino acids, such as from amino acid (aa) 1 to 210 and aa 220 to 538 of SEQ ID NO:2. The invention encompasses other fragments, however, such as any fragment in the protein greater than 6, 7, 8, or 9 amino acids.

- By "variants" is intended proteins or polypeptides having an amino acid sequence
- 20 that is at least about 45%, 55%, 65%, preferably about 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, or 98% identical to the amino acid sequence of SEQ ID NO:2 or 4. Variants also include polypeptides encoded by the cDNA insert of the plasmid deposited with ATCC as Accession Number _____, or polypeptides encoded by a nucleic acid molecule that hybridizes to the nucleic acid molecule of SEQ ID NO:1 or 3, or a
- 25 complement thereof, under stringent conditions. Such variants generally retain the functional activity of the IL-9/IL-2 receptor-like proteins of the invention. Variants include polypeptides that differ in amino acid sequence due to natural allelic variation or mutagenesis.

- The invention also provides IL-9/IL-2 receptor-like chimeric or fusion proteins.
- 30 As used herein, an IL-9/IL-2 receptor-like "chimeric protein" or "fusion protein" comprises an IL-9/IL-2 receptor-like polypeptide operably linked to a non-IL-9/IL-2

receptor-like polypeptide. A "IL-9/IL-2 receptor-like polypeptide" refers to a polypeptide having an amino acid sequence corresponding to an IL-9/IL-2 receptor-like protein, whereas a "non-IL-9/IL-2 receptor-like polypeptide" refers to a polypeptide having an amino acid sequence corresponding to a protein that is not substantially identical to the IL-9/IL-2 receptor-like protein, e.g., a protein that is different from the IL-9/IL-2 receptor-like protein and which is derived from the same or a different organism. Within an IL-9/IL-2 receptor-like fusion protein, the IL-9/IL-2 receptor-like polypeptide can correspond to all or a portion of an IL-9/IL-2 receptor-like protein, preferably at least one biologically active portion of an IL-9/IL-2 receptor-like protein. Within the fusion protein, the term "operably linked" is intended to indicate that the IL-9/IL-2 receptor-like polypeptide and the non-IL-9/IL-2 receptor-like polypeptide are fused in-frame to each other. The non-IL-9/IL-2 receptor-like polypeptide can be fused to the N-terminus or C-terminus of the IL-9/IL-2 receptor-like polypeptide.

One useful fusion protein is a GST-IL-9/IL-2 receptor-like fusion protein in which the IL-9/IL-2 receptor-like sequences are fused to the C-terminus of the GST sequences. Such fusion proteins can facilitate the purification of recombinant IL-9/IL-2 receptor-like proteins.

In yet another embodiment, the fusion protein is an IL-9/IL-2 receptor-like-immunoglobulin fusion protein in which all or part of an IL-9/IL-2 receptor-like protein is fused to sequences derived from a member of the immunoglobulin protein family. The IL-9/IL-2 receptor-like-immunoglobulin fusion proteins of the invention can be incorporated into pharmaceutical compositions and administered to a subject to inhibit an interaction between an IL-9/IL-2 receptor-like ligand and an IL-9/IL-2 receptor-like protein on the surface of a cell, thereby suppressing IL-9/IL-2 receptor-like-mediated signal transduction *in vivo*. The IL-9/IL-2 receptor-like-immunoglobulin fusion proteins can be used to affect the bioavailability of an IL-9/IL-2 receptor-like cognate ligand. Inhibition of the IL-9/IL-2 receptor-like ligand/IL-9/IL-2 receptor-like interaction may be useful therapeutically, both for treating proliferative and differentiative disorders and for modulating (e.g., promoting or inhibiting) cell survival. Moreover, the IL-9/IL-2 receptor-like-immunoglobulin fusion proteins of the invention can be used as immunogens to produce anti-IL-9/IL-2 receptor-like antibodies in a subject, to purify IL-

9/IL-2 receptor-like ligands, and in screening assays to identify molecules that inhibit the interaction of an IL-9/IL-2 receptor-like protein with an IL-9/IL-2 receptor-like ligand.

Preferably, an IL-9/IL-2 receptor-like chimeric or fusion protein of the invention is produced by standard recombinant DNA techniques. For example, DNA fragments
5 coding for the different polypeptide sequences may be ligated together in-frame, or the fusion gene can be synthesized, such as with automated DNA synthesizers.

Alternatively, PCR amplification of gene fragments can be carried out using anchor primers that give rise to complementary overhangs between two consecutive gene fragments, which can subsequently be annealed and reamplified to generate a chimeric
10 gene sequence (see, e.g., Ausubel *et al.*, eds. (1995) *Current Protocols in Molecular Biology*) (Greene Publishing and Wiley-Interscience, NY). Moreover, an IL-9/IL-2 receptor-like-encoding nucleic acid can be cloned into a commercially available expression vector such that it is linked in-frame to an existing fusion moiety.

Variants of the IL-9/IL-2 receptor-like proteins can function as either IL-9/IL-2
15 receptor-like agonists (mimetics) or as IL-9/IL-2 receptor-like antagonists. Variants of the IL-9/IL-2 receptor-like protein can be generated by mutagenesis, e.g., discrete point mutation or truncation of the IL-9/IL-2 receptor-like protein. An agonist of the IL-9/IL-2 receptor-like protein can retain substantially the same, or a subset, of the biological activities of the naturally occurring form of the IL-9/IL-2 receptor-like protein. An
20 antagonist of the IL-9/IL-2 receptor-like protein can inhibit one or more of the activities of the naturally occurring form of the IL-9/IL-2 receptor-like protein by, for example, competitively binding to a downstream or upstream member of a cellular signaling cascade that includes the IL-9/IL-2 receptor-like protein. Thus, specific biological effects can be elicited by treatment with a variant of limited function. Treatment of a subject
25 with a variant having a subset of the biological activities of the naturally occurring form of the protein can have fewer side effects in a subject relative to treatment with the naturally occurring form of the IL-9/IL-2 receptor-like proteins.

Variants of an IL-9/IL-2 receptor-like protein that function as either IL-9/IL-2 receptor-like agonists or as IL-9/IL-2 receptor-like antagonists can be identified by
30 screening combinatorial libraries of mutants, e.g., truncation mutants, of an IL-9/IL-2 receptor-like protein for IL-9/IL-2 receptor-like protein agonist or antagonist activity. In

one embodiment, a variegated library of IL-9/IL-2 receptor-like variants is generated by combinatorial mutagenesis at the nucleic acid level and is encoded by a variegated gene library. A variegated library of IL-9/IL-2 receptor-like variants can be produced by, for example, enzymatically ligating a mixture of synthetic oligonucleotides into gene
5 sequences such that a degenerate set of potential IL-9/IL-2 receptor-like sequences is expressible as individual polypeptides, or alternatively, as a set of larger fusion proteins (e.g., for phage display) containing the set of IL-9/IL-2 receptor-like sequences therein. There are a variety of methods that can be used to produce libraries of potential IL-9/IL-2 receptor-like variants from a degenerate oligonucleotide sequence. Chemical synthesis of
10 a degenerate gene sequence can be performed in an automatic DNA synthesizer, and the synthetic gene then ligated into an appropriate expression vector. Use of a degenerate set of genes allows for the provision, in one mixture, of all of the sequences encoding the desired set of potential IL-9/IL-2 receptor-like sequences. Methods for synthesizing degenerate oligonucleotides are known in the art (*see, e.g., Narang (1983) Tetrahedron*
15 39:3; Itakura *et al.* (1984) *Annu. Rev. Biochem.* 53:323; Itakura *et al.* (1984) *Science* 198:1056; Ike *et al.* (1983) *Nucleic Acid Res.* 11:477).

In addition, libraries of fragments of an IL-9/IL-2 receptor-like protein coding sequence can be used to generate a variegated population of IL-9/IL-2 receptor-like fragments for screening and subsequent selection of variants of an IL-9/IL-2 receptor-like
20 protein. In one embodiment, a library of coding sequence fragments can be generated by treating a double-stranded PCR fragment of an IL-9/IL-2 receptor-like coding sequence with a nuclease under conditions wherein nicking occurs only about once per molecule, denaturing the double-stranded DNA, renaturing the DNA to form double-stranded DNA which can include sense/antisense pairs from different nicked products, removing single-
25 stranded portions from reformed duplexes by treatment with S1 nuclease, and ligating the resulting fragment library into an expression vector. By this method, one can derive an expression library that encodes N-terminal and internal fragments of various sizes of the IL-9/IL-2 receptor-like protein.

Several techniques are known in the art for screening gene products of
30 combinatorial libraries made by point mutations or truncation and for screening cDNA libraries for gene products having a selected property. Such techniques are adaptable for

rapid screening of the gene libraries generated by the combinatorial mutagenesis of IL-9/IL-2 receptor-like proteins. The most widely used techniques, which are amenable to high through-put analysis, for screening large gene libraries typically include cloning the gene library into replicable expression vectors, transforming appropriate cells with the
5 resulting library of vectors, and expressing the combinatorial genes under conditions in which detection of a desired activity facilitates isolation of the vector encoding the gene whose product was detected. Recursive ensemble mutagenesis (REM), a technique that enhances the frequency of functional mutants in the libraries, can be used in combination with the screening assays to identify IL-9/IL-2 receptor-like variants (Arkin and Yourvan
10 (1992) *Proc. Natl. Acad. Sci. USA* 89:7811-7815; Delgrave *et al.* (1993) *Protein Engineering* 6(3):327-331).

An isolated IL-9/IL-2 receptor-like polypeptide of the invention can be used as an immunogen to generate antibodies that bind IL-9/IL-2 receptor-like proteins using standard techniques for polyclonal and monoclonal antibody preparation. The full-length
15 IL-9/IL-2 receptor-like protein can be used or, alternatively, the invention provides antigenic peptide fragments of IL-9/IL-2 receptor-like proteins for use as immunogens. The antigenic peptide of an IL-9/IL-2 receptor-like protein comprises at least 8, preferably 10, 15, 20, or 30 amino acid residues of the amino acid sequence shown in SEQ ID NO:2 or 4 and encompasses an epitope of an IL-9/IL-2 receptor-like protein such
20 that an antibody raised against the peptide forms a specific immune complex with the IL-9/IL-2 receptor-like protein. Preferred epitopes encompassed by the antigenic peptide are regions of a IL-9/IL-2 receptor-like protein that are located on the surface of the protein, e.g., hydrophilic regions.

Accordingly, another aspect of the invention pertains to anti-IL-9/IL-2 receptor-
25 like polyclonal and monoclonal antibodies that bind an IL-9/IL-2 receptor-like protein. Polyclonal anti-IL-9/IL-2 receptor-like antibodies can be prepared by immunizing a suitable subject (e.g., rabbit, goat, mouse, or other mammal) with an IL-9/IL-2 receptor-like immunogen. The anti-IL-9/IL-2 receptor-like antibody titer in the immunized subject can be monitored over time by standard techniques, such as with an enzyme
30 linked immunosorbent assay (ELISA) using immobilized IL-9/IL-2 receptor-like protein. At an appropriate time after immunization, e.g., when the anti-IL-9/IL-2 receptor-like

antibody titers are highest, antibody-producing cells can be obtained from the subject and used to prepare monoclonal antibodies by standard techniques, such as the hybridoma technique originally described by Kohler and Milstein (1975) *Nature* 256:495-497, the human B cell hybridoma technique (Kozbor *et al.* (1983) *Immunol. Today* 4:72), the
5 EBV-hybridoma technique (Cole *et al.* (1985) in *Monoclonal Antibodies and Cancer Therapy*, ed. Reisfeld and Sell (Alan R. Liss, Inc., New York, NY), pp. 77-96) or trioma techniques. The technology for producing hybridomas is well known (*see generally* Coligan *et al.*, eds. (1994) *Current Protocols in Immunology* (John Wiley & Sons, Inc., New York, NY); Galfre *et al.* (1977) *Nature* 266:55052; Kenneth (1980) in *Monoclonal*
10 *Antibodies: A New Dimension In Biological Analyses* (Plenum Publishing Corp., NY; and Lerner (1981) *Yale J. Biol. Med.*, 54:387-402).

Alternative to preparing monoclonal antibody-secreting hybridomas, a monoclonal anti-IL-9/IL-2 receptor-like antibody can be identified and isolated by screening a recombinant combinatorial immunoglobulin library (e.g., an antibody phage
15 display library) with an IL-9/IL-2 receptor-like protein to thereby isolate immunoglobulin library members that bind the IL-9/IL-2 receptor-like protein. Kits for generating and screening phage display libraries are commercially available (e.g., the Pharmacia *Recombinant Phage Antibody System*, Catalog No. 27-9400-01; and the Stratagene *SurfZAP™ Phage Display Kit*, Catalog No. 240612). Additionally, examples of methods
20 and reagents particularly amenable for use in generating and screening antibody display library can be found in, for example, U.S. Patent No. 5,223,409; PCT Publication Nos. WO 92/18619; WO 91/17271; WO 92/20791; WO 92/15679; 93/01288; WO 92/01047; 92/09690; and 90/02809; Fuchs *et al.* (1991) *Bio/Technology* 9:1370-1372; Hay *et al.* (1992) *Hum. Antibod. Hybridomas* 3:81-85; Huse *et al.* (1989) *Science* 246:1275-1281;
25 Griffiths *et al.* (1993) *EMBO J.* 12:725-734.

Additionally, recombinant anti-IL-9/IL-2 receptor-like antibodies, such as chimeric and humanized monoclonal antibodies, comprising both human and nonhuman portions, which can be made using standard recombinant DNA techniques, are within the scope of the invention. Such chimeric and humanized monoclonal antibodies can be
30 produced by recombinant DNA techniques known in the art, for example using methods described in PCT Publication Nos. WO 86101533 and WO 87/02671; European Patent

Application Nos. 184,187, 171,496, 125,023, and 173,494; U.S. Patent Nos. 4,816,567 and 5,225,539; European Patent Application 125,023; Better *et al.* (1988) *Science* 240:1041-1043; Liu *et al.* (1987) *Proc. Natl. Acad. Sci. USA* 84:3439-3443; Liu *et al.* (1987) *J. Immunol.* 139:3521-3526; Sun *et al.* (1987) *Proc. Natl. Acad. Sci. USA* 84:214-218; Nishimura *et al.* (1987) *Canc. Res.* 47:999-1005; Wood *et al.* (1985) *Nature* 314:446-449; Shaw *et al.* (1988) *J. Natl. Cancer Inst.* 80:1553-1559; Morrison (1985) *Science* 229:1202-1207; Oi *et al.* (1986) *Bio/Techniques* 4:214; Jones *et al.* (1986) *Nature* 321:552-525; Verhoeyan *et al.* (1988) *Science* 239:1534; and Beidler *et al.* (1988) *J. Immunol.* 141:4053-4060.

10 Completely human antibodies are particularly desirable for therapeutic treatment of human patients. Such antibodies can be produced using transgenic mice that are incapable of expressing endogenous immunoglobulin heavy and light chains genes, but which can express human heavy and light chain genes. *See*, for example, Lonberg and Huszar (1995) *Int. Rev. Immunol.* 13:65-93); and U.S. Patent Nos. 5,625,126; 5,633,425; 15 5,569,825; 5,661,016; and 5,545,806. In addition, companies such as Abgenix, Inc. (Freemont, CA), can be engaged to provide human antibodies directed against a selected antigen using technology similar to that described above.

Completely human antibodies that recognize a selected epitope can be generated using a technique referred to as "guided selection." In this approach a selected 20 non-human monoclonal antibody, e.g., a murine antibody, is used to guide the selection of a completely human antibody recognizing the same epitope. This technology is described by Jespers *et al.* (1994) *Bio/Technology* 12:899-903).

An anti-IL-9/IL-2 receptor-like antibody (e.g., monoclonal antibody) can be used to isolate IL-9/IL-2 receptor-like proteins by standard techniques, such as affinity 25 chromatography or immunoprecipitation. An anti-IL-9/IL-2 receptor-like antibody can facilitate the purification of natural IL-9/IL-2 receptor-like protein from cells and of recombinantly produced IL-9/IL-2 receptor-like protein expressed in host cells. Moreover, an anti-IL-9/IL-2 receptor-like antibody can be used to detect IL-9/IL-2 receptor-like protein (e.g., in a cellular lysate or cell supernatant) in order to evaluate the 30 abundance and pattern of expression of the IL-9/IL-2 receptor-like protein. Anti-IL-9/IL-2 receptor-like antibodies can be used diagnostically to monitor protein levels in tissue as

part of a clinical testing procedure, e.g., to, for example, determine the efficacy of a given treatment regimen. Detection can be facilitated by coupling the antibody to a detectable substance. Examples of detectable substances include various enzymes, prosthetic groups, fluorescent materials, luminescent materials, bioluminescent materials, and radioactive materials. Examples of suitable enzymes include horseradish peroxidase, alkaline phosphatase, β -galactosidase, or acetylcholinesterase; examples of suitable prosthetic group complexes include streptavidin/biotin and avidin/biotin; examples of suitable fluorescent materials include umbelliferone, fluorescein, fluorescein isothiocyanate, rhodamine, dichlorotriazinylamine fluorescein, dansyl chloride or phycoerythrin; an example of a luminescent material includes luminol; examples of bioluminescent materials include luciferase, luciferin, and aequorin; and examples of suitable radioactive material include ^{125}I , ^{131}I , ^{35}S , or ^3H .

Further, an antibody (or fragment thereof) may be conjugated to a therapeutic moiety such as a cytotoxin, a therapeutic agent or a radioactive metal ion. A cytotoxin or cytotoxic agent includes any agent that is detrimental to cells. Examples include taxol, cytochalasin B, gramicidin D, ethidium bromide, emetine, mitomycin, etoposide, tenoposide, vincristine, vinblastine, colchicin, doxorubicin, daunorubicin, dihydroxy anthracin dione, mitoxantrone, mithramycin, actinomycin D, 1-dehydrotestosterone, glucocorticoids, procaine, tetracaine, lidocaine, propranolol, and puromycin and analogs or homologs thereof. Therapeutic agents include, but are not limited to, antimetabolites (e.g., methotrexate, 6-mercaptopurine, 6-thioguanine, cytarabine, 5-fluorouracil decarbazine), alkylating agents (e.g., mechlorethamine, thioepa chlorambucil, melphalan, carmustine (BSNU) and lomustine (CCNU), cyclophosphamide, busulfan, dibromomannitol, streptozotocin, mitomycin C, and cis-dichlorodiamine platinum (II) (DDP) cisplatin), anthracyclines (e.g., daunorubicin (formerly daunomycin) and doxorubicin), antibiotics (e.g., dactinomycin (formerly actinomycin), bleomycin, mithramycin, and anthramycin (AMC)), and anti-mitotic agents (e.g., vincristine and vinblastine). The conjugates of the invention can be used for modifying a given biological response, the drug moiety is not to be construed as limited to classical chemical therapeutic agents. For example, the drug moiety may be a protein or polypeptide possessing a desired biological activity. Such proteins may include, for

example, a toxin such as abrin, ricin A, pseudomonas exotoxin, or diphtheria toxin; a protein such as tumor necrosis factor, alpha-interferon, beta-interferon, nerve growth factor, platelet derived growth factor, tissue plasminogen activator; or, biological response modifiers such as, for example, lymphokines, interleukin-1 ("IL-1"),

5 interleukin-2 ("IL-2"), interleukin-6 ("IL-6"), granulocyte macrophase colony stimulating factor ("GM-CSF"), granulocyte colony stimulating factor ("G-CSF"), or other growth factors.

Techniques for conjugating such therapeutic moiety to antibodies are well known, see, e.g., Arnon *et al.*, "Monoclonal Antibodies For Immunotargeting Of Drugs In Cancer
10 Therapy", in Monoclonal Antibodies And Cancer Therapy, Reisfeld *et al.* (eds.), pp. 243-56 (Alan R. Liss, Inc. 1985); Hellstrom *et al.*, "Antibodies For Drug Delivery", in Controlled Drug Delivery (2nd Ed.), Robinson *et al.* (eds.), pp. 623-53 (Marcel Dekker, Inc. 1987); Thorpe, "Antibody Carriers Of Cytotoxic Agents In Cancer Therapy: A Review", in Monoclonal Antibodies '84: Biological And Clinical Applications, Pinchera
15 *et al.* (eds.), pp. 475-506 (1985); "Analysis, Results, And Future Prospective Of The Therapeutic Use Of Radiolabeled Antibody In Cancer Therapy", in Monoclonal Antibodies For Cancer Detection And Therapy, Baldwin *et al.* (eds.), pp. 303-16 (Academic Press 1985), and Thorpe *et al.*, "The Preparation And Cytotoxic Properties Of Antibody-Toxin Conjugates", *Immunol. Rev.*, 62:119-58 (1982). Alternatively, an
20 antibody can be conjugated to a second antibody to form an antibody heteroconjugate as described by Segal in U.S. Patent No. 4,676,980.

III. Recombinant Expression Vectors and Host Cells

Another aspect of the invention pertains to vectors, preferably expression vectors,
25 containing a nucleic acid encoding an IL-9/IL-2 receptor-like protein (or a portion thereof). "Vector" refers to a nucleic acid molecule capable of transporting another nucleic acid to which it has been linked, such as a "plasmid", a circular double-stranded DNA loop into which additional DNA segments can be ligated, or a viral vector, where additional DNA segments can be ligated into the viral genome. The vectors are useful for
30 autonomous replication in a host cell or may be integrated into the genome of a host cell upon introduction into the host cell, and thereby are replicated along with the host

genome (e.g., nonepisomal mammalian vectors). Expression vectors are capable of directing the expression of genes to which they are operably linked. In general, expression vectors of utility in recombinant DNA techniques are often in the form of plasmids (vectors). However, the invention is intended to include such other forms of expression vectors, such as viral vectors (e.g., replication defective retroviruses, adenoviruses, and adeno-associated viruses), that serve equivalent functions.

The recombinant expression vectors of the invention comprise a nucleic acid of the invention in a form suitable for expression of the nucleic acid in a host cell. This means that the recombinant expression vectors include one or more regulatory sequences, selected on the basis of the host cells to be used for expression, operably linked to the nucleic acid sequence to be expressed. "Operably linked" is intended to mean that the nucleotide sequence of interest is linked to the regulatory sequence(s) in a manner that allows for expression of the nucleotide sequence (e.g., in an *in vitro* transcription/translation system or in a host cell when the vector is introduced into the host cell). The term "regulatory sequence" is intended to include promoters, enhancers, and other expression control elements (e.g., polyadenylation signals). See, for example, Goeddel (1990) in *Gene Expression Technology: Methods in Enzymology* 185 (Academic Press, San Diego, CA). Regulatory sequences include those that direct constitutive expression of a nucleotide sequence in many types of host cell and those that direct expression of the nucleotide sequence only in certain host cells (e.g., tissue-specific regulatory sequences). It will be appreciated by those skilled in the art that the design of the expression vector can depend on such factors as the choice of the host cell to be transformed, the level of expression of protein desired, etc. The expression vectors of the invention can be introduced into host cells to thereby produce proteins or peptides, including fusion proteins or peptides, encoded by nucleic acids as described herein (e.g., IL-9/IL-2 receptor-like proteins, mutant forms of IL-9/IL-2 receptor-like proteins, fusion proteins, etc.).

The recombinant expression vectors of the invention can be designed for expression of IL-9/IL-2 receptor-like protein in prokaryotic or eukaryotic host cells. Expression of proteins in prokaryotes is most often carried out in *E. coli* with vectors containing constitutive or inducible promoters directing the expression of either fusion or

nonfusion proteins. Fusion vectors add a number of amino acids to a protein encoded therein, usually to the amino terminus of the recombinant protein. Typical fusion expression vectors include pGEX (Pharmacia Biotech Inc; Smith and Johnson (1988) *Gene* 67:31-40), pMAL (New England Biolabs, Beverly, MA), and pRIT5 (Pharmacia, Piscataway, NJ) which fuse glutathione S-transferase (GST), maltose E binding protein, or protein A, respectively, to the target recombinant protein. Examples of suitable inducible nonfusion *E. coli* expression vectors include pTrc (Amann *et al.* (1988) *Gene* 69:301-315) and pET 11d (Studier *et al.* (1990) in *Gene Expression Technology: Methods in Enzymology* 185 (Academic Press, San Diego, CA), pp. 60-89). Strategies to maximize recombinant protein expression in *E. coli* can be found in Gottesman (1990) in *Gene Expression Technology: Methods in Enzymology* 185 (Academic Press, CA), pp. 119-128 and Wada *et al.* (1992) *Nucleic Acids Res.* 20:2111-2118. Target gene expression from the pTrc vector relies on host RNA polymerase transcription from a hybrid trp-lac fusion promoter.

Suitable eukaryotic host cells include insect cells (examples of Baculovirus vectors available for expression of proteins in cultured insect cells (e.g., Sf 9 cells) include the pAc series (Smith *et al.* (1983) *Mol. Cell Biol.* 3:2156-2165) and the pVL series (Lucklow and Summers (1989) *Virology* 170:31-39)); yeast cells (examples of vectors for expression in yeast *S. cerevisiae* include pYepSec1 (Baldari *et al.* (1987) *EMBO J.* 6:229-234), pMFa (Kurjan and Herskowitz (1982) *Cell* 30:933-943), pJRY88 (Schultz *et al.* (1987) *Gene* 54:113-123), pYES2 (Invitrogen Corporation, San Diego, CA), and pPicZ (Invitrogen Corporation, San Diego, CA)); or mammalian cells (mammalian expression vectors include pCDM8 (Seed (1987) *Nature* 329:840) and pMT2PC (Kaufman *et al.* (1987) *EMBO J.* 6:187:195)). Suitable mammalian cells include Chinese hamster ovary cells (CHO) or COS cells. In mammalian cells, the expression vector's control functions are often provided by viral regulatory elements. For example, commonly used promoters are derived from polyoma, Adenovirus 2, cytomegalovirus, and Simian Virus 40. For other suitable expression systems for both prokaryotic and eukaryotic cells, *see* chapters 16 and 17 of Sambrook *et al.* (1989) *Molecular cloning: A Laboratory Manual* (2d ed., Cold Spring Harbor Laboratory Press, Plainview, NY). *See*, Goeddel (1990) in *Gene Expression Technology: Methods in*

Enzymology 185 (Academic Press, San Diego, CA). Alternatively, the recombinant expression vector can be transcribed and translated *in vitro*, for example using T7 promoter regulatory sequences and T7 polymerase.

The terms "host cell" and "recombinant host cell" are used interchangeably
5 herein. It is understood that such terms refer not only to the particular subject cell but to the progeny or potential progeny of such a cell. Because certain modifications may occur in succeeding generations due to either mutation or environmental influences, such progeny may not, in fact, be identical to the parent cell but are still included within the scope of the term as used herein.

10 In one embodiment, the expression vector is a recombinant mammalian expression vector that comprises tissue-specific regulatory elements that direct expression of the nucleic acid preferentially in a particular cell type. Suitable tissue-specific promoters include the albumin promoter (liver-specific; Pinkert *et al.* (1987) *Genes Dev.* 1:268-277), lymphoid-specific promoters (Calame and Eaton (1988) *Adv.*
15 *Immunol.* 43:235-275), in particular promoters of T cell receptors (Winoto and Baltimore (1989) *EMBO J.* 8:729-733) and immunoglobulins (Banerji *et al.* (1983) *Cell* 33:729-740; Queen and Baltimore (1983) *Cell* 33:741-748), neuron-specific promoters (e.g., the neurofilament promoter; Byrne and Ruddle (1989) *Proc. Natl. Acad. Sci. USA* 86:5473-5477), pancreas-specific promoters (Edlund *et al.* (1985) *Science* 230:912-916), and
20 mammary gland-specific promoters (e.g., milk whey promoter; U.S. Patent No. 4,873,316 and European Application Patent Publication No. 264,166). Developmentally-regulated promoters are also encompassed, for example the murine homeobox (Hox) promoter (Kessel and Gruss (1990) *Science* 249:374-379), the α -fetoprotein promoter (Campes and Tilghman (1989) *Genes Dev.* 3:537-546), and the like.

25 The invention further provides a recombinant expression vector comprising a DNA molecule of the invention cloned into the expression vector in an antisense orientation. That is, the DNA molecule is operably linked to a regulatory sequence in a manner that allows for expression (by transcription of the DNA molecule) of an RNA molecule that is antisense to IL-9/IL-2 receptor-like mRNA. Regulatory sequences
30 operably linked to a nucleic acid cloned in the antisense orientation can be chosen to direct the continuous expression of the antisense RNA molecule in a variety of cell types,

for instance viral promoters and/or enhancers, or regulatory sequences can be chosen to direct constitutive, tissue-specific, or cell-type-specific expression of antisense RNA. The antisense expression vector can be in the form of a recombinant plasmid, phagemid, or attenuated virus in which antisense nucleic acids are produced under the control of a high efficiency regulatory region, the activity of which can be determined by the cell type into which the vector is introduced. For a discussion of the regulation of gene expression using antisense genes *see* Weintraub *et al.* (1986) *Reviews - Trends in Genetics*, Vol. 1(1).

Vector DNA can be introduced into prokaryotic or eukaryotic cells via conventional transformation or transfection techniques. As used herein, the terms "transformation" and "transfection" are intended to refer to a variety of art-recognized techniques for introducing foreign nucleic acid (e.g., DNA) into a host cell, including calcium phosphate or calcium chloride co-precipitation, DEAE-dextran-mediated transfection, lipofection, or electroporation. Suitable methods for transforming or transfecting host cells can be found in Sambrook *et al.* (1989) *Molecular Cloning: A Laboratory Manual* (2d ed., Cold Spring Harbor Laboratory Press, Plainview, NY) and other laboratory manuals.

For stable transfection of mammalian cells, it is known that, depending upon the expression vector and transfection technique used, only a small fraction of cells may integrate the foreign DNA into their genome. In order to identify and select these integrants, a gene that encodes a selectable marker (e.g., for resistance to antibiotics) is generally introduced into the host cells along with the gene of interest. Preferred selectable markers include those which confer resistance to drugs, such as G418, hygromycin, and methotrexate. Nucleic acid encoding a selectable marker can be introduced into a host cell on the same vector as that encoding an IL-9/IL-2 receptor-like protein or can be introduced on a separate vector. Cells stably transfected with the introduced nucleic acid can be identified by drug selection (e.g., cells that have incorporated the selectable marker gene will survive, while the other cells die).

A host cell of the invention, such as a prokaryotic or eukaryotic host cell in culture, can be used to produce (i.e., express) IL-9/IL-2 receptor-like protein. Accordingly, the invention further provides methods for producing IL-9/IL-2 receptor-

like protein using the host cells of the invention. In one embodiment, the method comprises culturing the host cell of the invention, into which a recombinant expression vector encoding an IL-9/IL-2 receptor-like protein has been introduced, in a suitable medium such that IL-9/IL-2 receptor-like protein is produced. In another embodiment, the method further comprises isolating IL-9/IL-2 receptor-like protein from the medium or the host cell.

The host cells of the invention can also be used to produce nonhuman transgenic animals. For example, in one embodiment, a host cell of the invention is a fertilized oocyte or an embryonic stem cell into which IL-9/IL-2 receptor-like-coding sequences have been introduced. Such host cells can then be used to create nonhuman transgenic animals in which exogenous IL-9/IL-2 receptor-like sequences have been introduced into their genome or homologous recombinant animals in which endogenous IL-9/IL-2 receptor-like sequences have been altered. Such animals are useful for studying the function and/or activity of IL-9/IL-2 receptor-like genes and proteins and for identifying and/or evaluating modulators of IL-9/IL-2 receptor-like activity. As used herein, a "transgenic animal" is a nonhuman animal, preferably a mammal, more preferably a rodent such as a rat or mouse, in which one or more of the cells of the animal includes a transgene. Other examples of transgenic animals include nonhuman primates, sheep, dogs, cows, goats, chickens, amphibians, etc. A transgene is exogenous DNA that is integrated into the genome of a cell from which a transgenic animal develops and which remains in the genome of the mature animal, thereby directing the expression of an encoded gene product in one or more cell types or tissues of the transgenic animal. As used herein, a "homologous recombinant animal" is a nonhuman animal, preferably a mammal, more preferably a mouse, in which an endogenous IL-9/IL-2 receptor-like gene has been altered by homologous recombination between the endogenous gene and an exogenous DNA molecule introduced into a cell of the animal, e.g., an embryonic cell of the animal, prior to development of the animal.

A transgenic animal of the invention can be created by introducing IL-9/IL-2 receptor-like-encoding nucleic acid into the male pronuclei of a fertilized oocyte, e.g., by microinjection, retroviral infection, and allowing the oocyte to develop in a pseudopregnant female foster animal. The IL-9/IL-2 receptor-like cDNA sequence can

be introduced as a transgene into the genome of a nonhuman animal. Alternatively, a
homologue of the mouse IL-9/IL-2 receptor-like gene can be isolated based on
hybridization and used as a transgene. Intronic sequences and polyadenylation signals
can also be included in the transgene to increase the efficiency of expression of the
5 transgene. A tissue-specific regulatory sequence(s) can be operably linked to the IL-
9/IL-2 receptor-like transgene to direct expression of IL-9/IL-2 receptor-like protein to
particular cells. Methods for generating transgenic animals via embryo manipulation and
microinjection, particularly animals such as mice, have become conventional in the art
and are described, for example, in U.S. Patent Nos. 4,736,866, 4,870,009, and 4,873,191
10 and in Hogan (1986) *Manipulating the Mouse Embryo* (Cold Spring Harbor Laboratory
Press, Cold Spring Harbor, NY, 1986). Similar methods are used for production of other
transgenic animals. A transgenic founder animal can be identified based upon the
presence of the IL-9/IL-2 receptor-like transgene in its genome and/or expression of IL-
9/IL-2 receptor-like mRNA in tissues or cells of the animals. A transgenic founder
15 animal can then be used to breed additional animals carrying the transgene. Moreover,
transgenic animals carrying a transgene encoding IL-9/IL-2 receptor-like gene can further
be bred to other transgenic animals carrying other transgenes.

To create a homologous recombinant animal, one prepares a vector containing at
least a portion of an IL-9/IL-2 receptor-like gene or a homolog of the gene into which a
20 deletion, addition, or substitution has been introduced to thereby alter, e.g., functionally
disrupt, the IL-9/IL-2 receptor-like gene. In a preferred embodiment, the vector is
designed such that, upon homologous recombination, the endogenous IL-9/IL-2 receptor-
like gene is functionally disrupted (i.e., no longer encodes a functional protein; also
referred to as a "knock out" vector). Alternatively, the vector can be designed such that,
25 upon homologous recombination, the endogenous IL-9/IL-2 receptor-like gene is mutated
or otherwise altered but still encodes functional protein (e.g., the upstream regulatory
region can be altered to thereby alter the expression of the endogenous IL-9/IL-2
receptor-like protein). In the homologous recombination vector, the altered portion of the
IL-9/IL-2 receptor-like gene is flanked at its 5' and 3' ends by additional nucleic acid of
30 the IL-9/IL-2 receptor-like gene to allow for homologous recombination to occur
between the exogenous IL-9/IL-2 receptor-like gene carried by the vector and an

endogenous IL-9/IL-2 receptor-like gene in an embryonic stem cell. The additional flanking IL-9/IL-2 receptor-like nucleic acid is of sufficient length for successful homologous recombination with the endogenous gene. Typically, several kilobases of flanking DNA (both at the 5' and 3' ends) are included in the vector (*see, e.g., Thomas and Capecchi (1987) Cell 51:503* for a description of homologous recombination vectors). The vector is introduced into an embryonic stem cell line (e.g., by electroporation), and cells in which the introduced IL-9/IL-2 receptor-like gene has homologously recombined with the endogenous IL-9/IL-2 receptor-like gene are selected (*see, e.g., Li et al. (1992) Cell 69:915*). The selected cells are then injected into a blastocyst of an animal (e.g., a mouse) to form aggregation chimeras (*see, e.g., Bradley (1987) in Teratocarcinomas and Embryonic Stem Cells: A Practical Approach, ed. Robertson (IRL, Oxford pp. 113-152)*). A chimeric embryo can then be implanted into a suitable pseudopregnant female foster animal and the embryo brought to term. Progeny harboring the homologously recombined DNA in their germ cells can be used to breed animals in which all cells of the animal contain the homologously recombined DNA by germline transmission of the transgene. Methods for constructing homologous recombination vectors and homologous recombinant animals are described further in Bradley (1991) *Current Opinion in Bio/Technology* 2:823-829 and in PCT Publication Nos. WO 90/11354, WO 91/01140, WO 92/0968, and WO 93/04169.

In another embodiment, transgenic nonhuman animals containing selected systems that allow for regulated expression of the transgene can be produced. One example of such a system is the *cre/loxP* recombinase system of bacteriophage P1. For a description of the *cre/loxP* recombinase system, *see, e.g., Lakso et al. (1992) Proc. Natl. Acad. Sci. USA 89:6232-6236*. Another example of a recombinase system is the FLP recombinase system of *Saccharomyces cerevisiae* (O'Gorman *et al. (1991) Science* 251:1351-1355). If a *cre/loxP* recombinase system is used to regulate expression of the transgene, animals containing transgenes encoding both the *Cre* recombinase and a selected protein are required. Such animals can be provided through the construction of "double" transgenic animals, e.g., by mating two transgenic animals, one containing a transgene encoding a selected protein and the other containing a transgene encoding a recombinase.

Clones of the nonhuman transgenic animals described herein can also be produced according to the methods described in Wilmot *et al.* (1997) *Nature* 385:810-813 and PCT Publication Nos. WO 97/07668 and WO 97/07669.

5 IV. Pharmaceutical Compositions

 The IL-9/IL-2 receptor-like nucleic acid molecules, IL-9/IL-2 receptor-like proteins, and anti-IL-9/IL-2 receptor-like antibodies (also referred to herein as "active compounds") of the invention can be incorporated into pharmaceutical compositions suitable for administration. Such compositions typically comprise the nucleic acid
10 molecule, protein, or antibody and a pharmaceutically acceptable carrier. As used herein the language "pharmaceutically acceptable carrier" is intended to include any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like, compatible with pharmaceutical administration. The use of such media and agents for pharmaceutically active substances is well known
15 in the art. Except insofar as any conventional media or agent is incompatible with the active compound, use thereof in the compositions is contemplated. Supplementary active compounds can also be incorporated into the compositions.

 The compositions of the invention are useful to treat any of the disorders discussed herein. The compositions are provided in therapeutically effective amounts.
20 By "therapeutically effective amounts" is intended an amount sufficient to modulate the desired response. As defined herein, a therapeutically effective amount of protein or polypeptide (i.e., an effective dosage) ranges from about 0.001 to 30 mg/kg body weight, preferably about 0.01 to 25 mg/kg body weight, more preferably about 0.1 to 20 mg/kg body weight, and even more preferably about 1 to 10 mg/kg, 2 to 9 mg/kg, 3 to 8 mg/kg,
25 4 to 7 mg/kg, or 5 to 6 mg/kg body weight.

 The skilled artisan will appreciate that certain factors may influence the dosage required to effectively treat a subject, including but not limited to the severity of the disease or disorder, previous treatments, the general health and/or age of the subject, and other diseases present. Moreover, treatment of a subject with a therapeutically effective
30 amount of a protein, polypeptide, or antibody can include a single treatment or, preferably, can include a series of treatments. In a preferred example, a subject is treated

with antibody, protein, or polypeptide in the range of between about 0.1 to 20 mg/kg body weight, one time per week for between about 1 to 10 weeks, preferably between 2 to 8 weeks, more preferably between about 3 to 7 weeks, and even more preferably for about 4, 5, or 6 weeks. It will also be appreciated that the effective dosage of antibody, protein, or polypeptide used for treatment may increase or decrease over the course of a particular treatment. Changes in dosage may result and become apparent from the results of diagnostic assays as described herein.

The present invention encompasses agents which modulate expression or activity. An agent may, for example, be a small molecule. For example, such small molecules include, but are not limited to, peptides, peptidomimetics, amino acids, amino acid analogs, polynucleotides, polynucleotide analogs, nucleotides, nucleotide analogs, organic or inorganic compounds (i.e., including heteroorganic and organometallic compounds) having a molecular weight less than about 10,000 grams per mole, organic or inorganic compounds having a molecular weight less than about 5,000 grams per mole, organic or inorganic compounds having a molecular weight less than about 1,000 grams per mole, organic or inorganic compounds having a molecular weight less than about 500 grams per mole, and salts, esters, and other pharmaceutically acceptable forms of such compounds.

It is understood that appropriate doses of small molecule agents depends upon a number of factors within the knowledge of the ordinarily skilled physician, veterinarian, or researcher. The dose(s) of the small molecule will vary, for example, depending upon the identity, size, and condition of the subject or sample being treated, further depending upon the route by which the composition is to be administered, if applicable, and the effect which the practitioner desires the small molecule to have upon the nucleic acid or polypeptide of the invention. Exemplary doses include milligram or microgram amounts of the small molecule per kilogram of subject or sample weight (e.g., about 1 microgram per kilogram to about 500 milligrams per kilogram, about 100 micrograms per kilogram to about 5 milligrams per kilogram, or about 1 microgram per kilogram to about 50 micrograms per kilogram. It is furthermore understood that appropriate doses of a small molecule depend upon the potency of the small molecule with respect to the expression or activity to be modulated. Such appropriate doses may be determined using the assays

described herein. When one or more of these small molecules is to be administered to an animal (e.g., a human) in order to modulate expression or activity of a polypeptide or nucleic acid of the invention, a physician, veterinarian, or researcher may, for example, prescribe a relatively low dose at first, subsequently increasing the dose until an
5 appropriate response is obtained. In addition, it is understood that the specific dose level for any particular animal subject will depend upon a variety of factors including the activity of the specific compound employed, the age, body weight, general health, gender, and diet of the subject, the time of administration, the route of administration, the rate of excretion, any drug combination, and the degree of expression or activity to be
10 modulated.

A pharmaceutical composition of the invention is formulated to be compatible with its intended route of administration. Examples of routes of administration include parenteral, e.g., intravenous, intradermal, subcutaneous, oral (e.g., inhalation), transdermal (topical), transmucosal, and rectal administration. Solutions or suspensions
15 used for parenteral, intradermal, or subcutaneous application can include the following components: a sterile diluent such as water for injection, saline solution, fixed oils, polyethylene glycols, glycerine, propylene glycol or other synthetic solvents; antibacterial agents such as benzyl alcohol or methyl parabens; antioxidants such as ascorbic acid or sodium bisulfite; chelating agents such as ethylenediaminetetraacetic
20 acid; buffers such as acetates, citrates or phosphates and agents for the adjustment of tonicity such as sodium chloride or dextrose. pH can be adjusted with acids or bases, such as hydrochloric acid or sodium hydroxide. The parenteral preparation can be enclosed in ampoules, disposable syringes, or multiple dose vials made of glass or plastic.

Pharmaceutical compositions suitable for injectable use include sterile aqueous
25 solutions (where water soluble) or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersions. For intravenous administration, suitable carriers include physiological saline, bacteriostatic water, Cremophor EL™ (BASF; Parsippany, NJ), or phosphate buffered saline (PBS). In all cases, the composition must be sterile and should be fluid to the extent that easy
30 syringability exists. It must be stable under the conditions of manufacture and storage and must be preserved against the contaminating action of microorganisms such as

bacteria and fungi. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, and liquid polyethylene glycol, and the like), and suitable mixtures thereof. The proper fluidity can be maintained, for example, by the use of a coating such as lecithin, by the maintenance
5 of the required particle size in the case of dispersion, and by the use of surfactants. Prevention of the action of microorganisms can be achieved by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, ascorbic acid, thimerosal, and the like. In many cases, it will be preferable to include isotonic agents, for example, sugars, polyalcohols such as mannitol, sorbitol, sodium chloride, in the
10 composition. Prolonged absorption of the injectable compositions can be brought about by including in the composition an agent that delays absorption, for example, aluminum monostearate and gelatin.

Sterile injectable solutions can be prepared by incorporating the active compound (e.g., an IL-9/IL-2 receptor-like protein or anti-IL-9/IL-2 receptor-like antibody) in the
15 required amount in an appropriate solvent with one or a combination of ingredients enumerated above, as required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the active compound into a sterile vehicle that contains a basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions,
20 the preferred methods of preparation are vacuum drying and freeze-drying, which yields a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof.

Oral compositions generally include an inert diluent or an edible carrier. They can be enclosed in gelatin capsules or compressed into tablets. For the purpose of oral
25 therapeutic administration, the active compound can be incorporated with excipients and used in the form of tablets, troches, or capsules. Oral compositions can also be prepared using a fluid carrier for use as a mouthwash, wherein the compound in the fluid carrier is applied orally and swished and expectorated or swallowed. Pharmaceutically compatible binding agents, and/or adjuvant materials can be included as part of the composition. The
30 tablets, pills, capsules, troches and the like can contain any of the following ingredients, or compounds of a similar nature: a binder such as microcrystalline cellulose, gum

tragacanth, or gelatin; an excipient such as starch or lactose, a disintegrating agent such as alginic acid, Primogel, or corn starch; a lubricant such as magnesium stearate or Sterotes; a glidant such as colloidal silicon dioxide; a sweetening agent such as sucrose or saccharin; or a flavoring agent such as peppermint, methyl salicylate, or orange flavoring.

- 5 For administration by inhalation, the compounds are delivered in the form of an aerosol spray from a pressurized container or dispenser that contains a suitable propellant, e.g., a gas such as carbon dioxide, or a nebulizer.

Systemic administration can also be by transmucosal or transdermal means. For transmucosal or transdermal administration, penetrants appropriate to the barrier to be
10 permeated are used in the formulation. Such penetrants are generally known in the art, and include, for example, for transmucosal administration, detergents, bile salts, and fusidic acid derivatives. Transmucosal administration can be accomplished through the use of nasal sprays or suppositories. For transdermal administration, the active
15 compounds are formulated into ointments, salves, gels, or creams as generally known in the art. The compounds can also be prepared in the form of suppositories (e.g., with conventional suppository bases such as cocoa butter and other glycerides) or retention enemas for rectal delivery.

In one embodiment, the active compounds are prepared with carriers that will protect the compound against rapid elimination from the body, such as a controlled
20 release formulation, including implants and microencapsulated delivery systems. Biodegradable, biocompatible polymers can be used, such as ethylene vinyl acetate, polyanhydrides, polyglycolic acid, collagen, polyorthoesters, and polylactic acid. Methods for preparation of such formulations will be apparent to those skilled in the art. The materials can also be obtained commercially from Alza Corporation and Nova
25 Pharmaceuticals, Inc. Liposomal suspensions (including liposomes targeted to infected cells with monoclonal antibodies to viral antigens) can also be used as pharmaceutically acceptable carriers. These can be prepared according to methods known to those skilled in the art, for example, as described in U.S. Patent No. 4,522,811.

It is especially advantageous to formulate oral or parenteral compositions in
30 dosage unit form for ease of administration and uniformity of dosage. Dosage unit form as used herein refers to physically discrete units suited as unitary dosages for the subject

to be treated with each unit containing a predetermined quantity of active compound calculated to produce the desired therapeutic effect in association with the required pharmaceutical carrier. Depending on the type and severity of the disease, about 1 µg/kg to about 15 mg/kg (e.g., 0.1 to 20 mg/kg) of antibody is an initial candidate dosage for administration to the patient, whether, for example, by one or more separate administrations, or by continuous infusion. A typical daily dosage might range from about 1 µg/kg to about 100 mg/kg or more, depending on the factors mentioned above. For repeated administrations over several days or longer, depending on the condition, the treatment is sustained until a desired suppression of disease symptoms occurs. However, other dosage regimens may be useful. The progress of this therapy is easily monitored by conventional techniques and assays. An exemplary dosing regimen is disclosed in WO 94/04188. The specification for the dosage unit forms of the invention are dictated by and directly dependent on the unique characteristics of the active compound and the particular therapeutic effect to be achieved, and the limitations inherent in the art of compounding such an active compound for the treatment of individuals.

The nucleic acid molecules of the invention can be inserted into vectors and used as gene therapy vectors. Gene therapy vectors can be delivered to a subject by, for example, intravenous injection, local administration (U.S. Patent 5,328,470), or by stereotactic injection (*see, e.g., Chen et al. (1994) Proc. Natl. Acad. Sci. USA 91:3054-3057*). The pharmaceutical preparation of the gene therapy vector can include the gene therapy vector in an acceptable diluent, or can comprise a slow release matrix in which the gene delivery vehicle is imbedded. Alternatively, where the complete gene delivery vector can be produced intact from recombinant cells, e.g., retroviral vectors, the pharmaceutical preparation can include one or more cells which produce the gene delivery system.

The pharmaceutical compositions can be included in a container, pack, or dispenser together with instructions for administration.

V. Uses and Methods of the Invention

The nucleic acid molecules, proteins, protein homologues, and antibodies described herein can be used in one or more of the following methods: (a) screening

assays; (b) detection assays (e.g., chromosomal mapping, tissue typing, forensic biology); (c) predictive medicine (e.g., diagnostic assays, prognostic assays, monitoring clinical trials, and pharmacogenomics); and (d) methods of treatment (e.g., therapeutic and prophylactic). The isolated nucleic acid molecules of the invention can be used to

5 express IL-9/IL-2 receptor-like protein (e.g., via a recombinant expression vector in a host cell in gene therapy applications), to detect IL-9/IL-2 receptor-like mRNA (e.g., in a biological sample) or a genetic lesion in an IL-9/IL-2 receptor-like gene, and to modulate IL-9/IL-2 receptor-like activity. In addition, the IL-9/IL-2 receptor-like proteins can be used to screen drugs or compounds that modulate the immune response as well as to treat

10 disorders characterized by insufficient or excessive production of IL-9/IL-2 receptor-like protein or production of IL-9/IL-2 receptor-like protein forms that have decreased or aberrant activity compared to IL-9/IL-2 receptor-like wild type protein. In addition, the anti-IL-9/IL-2 receptor-like antibodies of the invention can be used to detect and isolate IL-9/IL-2 receptor-like proteins and modulate IL-9/IL-2 receptor-like activity.

15

A. Screening Assays

The invention provides a method (also referred to herein as a "screening assay") for identifying modulators, i.e., candidate or test compounds or agents (e.g., peptides, peptidomimetics, small molecules, or other drugs) that bind to IL-9/IL-2 receptor-like

20 proteins or have a stimulatory or inhibitory effect on, for example, IL-9/IL-2 receptor-like expression or IL-9/IL-2 receptor-like activity.

The test compounds of the present invention can be obtained using any of the numerous approaches in combinatorial library methods known in the art, including biological libraries, spatially addressable parallel solid phase or solution phase libraries,

25 synthetic library methods requiring deconvolution, the "one-bead one-compound" library method, and synthetic library methods using affinity chromatography selection. The biological library approach is limited to peptide libraries, while the other four approaches are applicable to peptide, nonpeptide oligomer, or small molecule libraries of compounds (Lam (1997) *Anticancer Drug Des.* 12:145).

30 Examples of methods for the synthesis of molecular libraries can be found in the art, for example in: DeWitt *et al.* (1993) *Proc. Natl. Acad. Sci. USA* 90:6909; Erb *et al.*

(1994) *Proc. Natl. Acad. Sci. USA* 91:11422; Zuckermann *et al.* (1994). *J. Med. Chem.* 37:2678; Cho *et al.* (1993) *Science* 261:1303; Carrell *et al.* (1994) *Angew. Chem. Int. Ed. Engl.* 33:2059; Carell *et al.* (1994) *Angew. Chem. Int. Ed. Engl.* 33:2061; and Gallop *et al.* (1994) *J. Med. Chem.* 37:1233.

5 Libraries of compounds may be presented in solution (e.g., Houghten (1992) *Bio/Techniques* 13:412-421), or on beads (Lam (1991) *Nature* 354:82-84), chips (Fodor (1993) *Nature* 364:555-556), bacteria (U.S. Patent No. 5,223,409), spores (U.S. Patent Nos. 5,571,698; 5,403,484; and 5,223,409), plasmids (Cull *et al.* (1992) *Proc. Natl. Acad. Sci. USA* 89:1865-1869), or phage (Scott and Smith (1990) *Science* 249:386-390; Devlin
10 (1990) *Science* 249:404-406; Cwirla *et al.* (1990) *Proc. Natl. Acad. Sci. USA* 87:6378-6382; and Felici (1991) *J. Mol. Biol.* 222:301-310).

Determining the ability of the test compound to bind to the IL-9/IL-2 receptor-like protein can be accomplished, for example, by coupling the test compound with a radioisotope or enzymatic label such that binding of the test compound to the IL-9/IL-2
15 receptor-like protein or biologically active portion thereof can be determined by detecting the labeled compound in a complex. For example, test compounds can be labeled with ¹²⁵I, ³⁵S, ¹⁴C, or ³H, either directly or indirectly, and the radioisotope detected by direct counting of radioemission or by scintillation counting. Alternatively, test compounds can be enzymatically labeled with, for example, horseradish peroxidase, alkaline
20 phosphatase, or luciferase, and the enzymatic label detected by determination of conversion of an appropriate substrate to product.

In a similar manner, one may determine the ability of the IL-9/IL-2 receptor-like protein to bind to or interact with an IL-9/IL-2 receptor-like target molecule. By "target molecule" is intended a molecule with which an IL-9/IL-2 receptor-like protein binds or
25 interacts in nature. In a preferred embodiment, the ability of the IL-9/IL-2 receptor-like protein to bind to or interact with an IL-9/IL-2 receptor-like target molecule can be determined by monitoring the activity of the target molecule. For example, the activity of the target molecule can be monitored by detecting induction of a cellular second messenger of the target (e.g., intracellular Ca²⁺, diacylglycerol, IP3, etc.), detecting
30 catalytic/enzymatic activity of the target on an appropriate substrate, detecting the induction of a reporter gene (e.g., an IL-9/IL-2 receptor-like-responsive regulatory

element operably linked to a nucleic acid encoding a detectable marker, e.g. luciferase), or detecting a cellular response, for example, cellular differentiation or cell proliferation.

In yet another embodiment, an assay of the present invention is a cell-free assay comprising contacting an IL-9/IL-2 receptor-like protein or biologically active portion thereof with a test compound and determining the ability of the test compound to bind to the IL-9/IL-2 receptor-like protein or biologically active portion thereof. Binding of the test compound to the IL-9/IL-2 receptor-like protein can be determined either directly or indirectly as described above. In a preferred embodiment, the assay includes contacting the IL-9/IL-2 receptor-like protein or biologically active portion thereof with a known compound that binds IL-9/IL-2 receptor-like protein to form an assay mixture, contacting the assay mixture with a test compound, and determining the ability of the test compound to preferentially bind to IL-9/IL-2 receptor-like protein or biologically active portion thereof as compared to the known compound.

In another embodiment, an assay is a cell-free assay comprising contacting IL-9/IL-2 receptor-like protein or biologically active portion thereof with a test compound and determining the ability of the test compound to modulate (e.g., stimulate or inhibit) the activity of the IL-9/IL-2 receptor-like protein or biologically active portion thereof. Determining the ability of the test compound to modulate the activity of an IL-9/IL-2 receptor-like protein can be accomplished, for example, by determining the ability of the IL-9/IL-2 receptor-like protein to bind to an IL-9/IL-2 receptor-like target molecule as described above for determining direct binding. In an alternative embodiment, determining the ability of the test compound to modulate the activity of an IL-9/IL-2 receptor-like protein can be accomplished by determining the ability of the IL-9/IL-2 receptor-like protein to further modulate an IL-9/IL-2 receptor-like target molecule. For example, the catalytic/enzymatic activity of the target molecule on an appropriate substrate can be determined as previously described.

In yet another embodiment, the cell-free assay comprises contacting the IL-9/IL-2 receptor-like protein or biologically active portion thereof with a known compound that binds an IL-9/IL-2 receptor-like protein to form an assay mixture, contacting the assay mixture with a test compound, and determining the ability of the test compound to

preferentially bind to or modulate the activity of an IL-9/IL-2 receptor-like target molecule.

In the above-mentioned assays, it may be desirable to immobilize either an IL-9/IL-2 receptor-like protein or its target molecule to facilitate separation of complexed
5 from uncomplexed forms of one or both of the proteins, as well as to accommodate automation of the assay. In one embodiment, a fusion protein can be provided that adds a domain that allows one or both of the proteins to be bound to a matrix. For example, glutathione-S-transferase/IL-9/IL-2 receptor-like fusion proteins or glutathione-S-transferase/target fusion proteins can be adsorbed onto glutathione sepharose beads
10 (Sigma Chemical, St. Louis, MO) or glutathione-derivatized microtitre plates, which are then combined with the test compound or the test compound and either the nonadsorbed target protein or IL-9/IL-2 receptor-like protein, and the mixture incubated under conditions conducive to complex formation (e.g., at physiological conditions for salt and pH). Following incubation, the beads or microtitre plate wells are washed to remove any
15 unbound components and complex formation is measured either directly or indirectly, for example, as described above. Alternatively, the complexes can be dissociated from the matrix, and the level of IL-9/IL-2 receptor-like binding or activity determined using standard techniques.

Other techniques for immobilizing proteins on matrices can also be used in the
20 screening assays of the invention. For example, either IL-9/IL-2 receptor-like protein or its target molecule can be immobilized utilizing conjugation of biotin and streptavidin. Biotinylated IL-9/IL-2 receptor-like molecules or target molecules can be prepared from biotin-NHS (N-hydroxy-succinimide) using techniques well known in the art (e.g., biotinylation kit, Pierce Chemicals, Rockford, IL), and immobilized in the wells of
25 streptavidin-coated 96-well plates (Pierce Chemicals). Alternatively, antibodies reactive with an IL-9/IL-2 receptor-like protein or target molecules but which do not interfere with binding of the IL-9/IL-2 receptor-like protein to its target molecule can be derivatized to the wells of the plate, and unbound target or IL-9/IL-2 receptor-like protein trapped in the wells by antibody conjugation. Methods for detecting such complexes, in
30 addition to those described above for the GST-immobilized complexes, include immunodetection of complexes using antibodies reactive with the IL-9/IL-2 receptor-like

protein or target molecule, as well as enzyme-linked assays that rely on detecting an enzymatic activity associated with the IL-9/IL-2 receptor-like protein or target molecule.

In another embodiment, modulators of IL-9/IL-2 receptor-like expression are identified in a method in which a cell is contacted with a candidate compound and the expression of IL-9/IL-2 receptor-like mRNA or protein in the cell is determined relative to expression of IL-9/IL-2 receptor-like mRNA or protein in a cell in the absence of the candidate compound. When expression is greater (statistically significantly greater) in the presence of the candidate compound than in its absence, the candidate compound is identified as a stimulator of IL-9/IL-2 receptor-like mRNA or protein expression.

Alternatively, when expression is less (statistically significantly less) in the presence of the candidate compound than in its absence, the candidate compound is identified as an inhibitor of IL-9/IL-2 receptor-like mRNA or protein expression. The level of IL-9/IL-2 receptor-like mRNA or protein expression in the cells can be determined by methods described herein for detecting IL-9/IL-2 receptor-like mRNA or protein.

In yet another aspect of the invention, the IL-9/IL-2 receptor-like proteins can be used as "bait proteins" in a two-hybrid assay or three-hybrid assay (*see, e.g.,* U.S. Patent No. 5,283,317; Zervos *et al.* (1993) *Cell* 72:223-232; Madura *et al.* (1993) *J. Biol. Chem.* 268:12046-12054; Bartel *et al.* (1993) *Bio/Techniques* 14:920-924; Iwabuchi *et al.* (1993) *Oncogene* 8:1693-1696; and PCT Publication No. WO 94/10300), to identify other proteins, which bind to or interact with IL-9/IL-2 receptor-like protein ("IL-9/IL-2 receptor-like-binding proteins" or "IL-9/IL-2 receptor-like-bp") and modulate IL-9/IL-2 receptor-like activity. Such IL-9/IL-2 receptor-like-binding proteins are also likely to be involved in the propagation of signals by the IL-9/IL-2 receptor-like proteins as, for example, upstream or downstream elements of the IL-9/IL-2 receptor-like pathway.

This invention further pertains to novel agents identified by the above-described screening assays and uses thereof for treatments as described herein.

B. Detection Assays

Portions or fragments of the cDNA sequences identified herein (and the corresponding complete gene sequences) can be used in numerous ways as polynucleotide reagents. For example, these sequences can be used to: (1) map their

respective genes on a chromosome; (2) identify an individual from a minute biological sample (tissue typing); and (3) aid in forensic identification of a biological sample. These applications are described in the subsections below.

5 1. Chromosome Mapping

 The isolated IL-9/IL-2 receptor-like gene sequences of the invention can be used to map their respective IL-9/IL-2 receptor-like genes on a chromosome, thereby facilitating the location of gene regions associated with genetic disease. Computer analysis of IL-9/IL-2 receptor-like sequences can be used to rapidly select PCR primers
10 (preferably 15-25 bp in length) that do not span more than one exon in the genomic DNA, thereby simplifying the amplification process. These primers can then be used for PCR screening of somatic cell hybrids containing individual human chromosomes. Only those hybrids containing the human gene corresponding to the IL-9/IL-2 receptor-like sequences will yield an amplified fragment.

15 Somatic cell hybrids are prepared by fusing somatic cells from different mammals (e.g., human and mouse cells). As hybrids of human and mouse cells grow and divide, they gradually lose human chromosomes in random order, but retain the mouse chromosomes. By using media in which mouse cells cannot grow (because they lack a particular enzyme), but in which human cells can, the one human chromosome that
20 contains the gene encoding the needed enzyme will be retained. By using various media, panels of hybrid cell lines can be established. Each cell line in a panel contains either a single human chromosome or a small number of human chromosomes, and a full set of mouse chromosomes, allowing easy mapping of individual genes to specific human chromosomes (D'Eustachio *et al.* (1983) *Science* 220:919-924). Somatic cell hybrids
25 containing only fragments of human chromosomes can also be produced by using human chromosomes with translocations and deletions.

 Other mapping strategies that can similarly be used to map an IL-9/IL-2 receptor-like sequence to its chromosome include *in situ* hybridization (described in Fan *et al.* (1990) *Proc. Natl. Acad. Sci. USA* 87:6223-27), pre-screening with labeled flow-sorted
30 chromosomes, and pre-selection by hybridization to chromosome specific cDNA libraries. Furthermore, fluorescence *in situ* hybridization (FISH) of a DNA sequence to a

metaphase chromosomal spread can be used to provide a precise chromosomal location in one step. For a review of this technique, see Verma *et al.* (1988) *Human Chromosomes: A Manual of Basic Techniques* (Pergamon Press, NY). The FISH technique can be used with a DNA sequence as short as 500 or 600 bases. However, clones larger than 1,000
5 bases have a higher likelihood of binding to a unique chromosomal location with sufficient signal intensity for simple detection. Preferably 1,000 bases, and more preferably 2,000 bases will suffice to get good results in a reasonable amount of time.

Reagents for chromosome mapping can be used individually to mark a single chromosome or a single site on that chromosome, or panels of reagents can be used for
10 marking multiple sites and/or multiple chromosomes. Reagents corresponding to noncoding regions of the genes actually are preferred for mapping purposes. Coding sequences are more likely to be conserved within gene families, thus increasing the chance of cross hybridizations during chromosomal mapping.

Another strategy to map the chromosomal location of IL-9/IL-2 receptor-like
15 genes uses IL-9/IL-2 receptor -like polypeptides and fragments and sequences of the present invention and antibodies specific thereto. This mapping can be carried out by specifically detecting the presence of a IL-9/IL-2 receptor -like polypeptide in members of a panel of somatic cell hybrids between cells of a first species of animal from which the protein originates and cells from a second species of animal, and then determining
20 which somatic cell hybrid(s) expresses the polypeptide and noting the chromosomes(s) from the first species of animal that it contains. For examples of this technique, see Pajunen *et al.* (1988) *Cytogenet. Cell. Genet.* 47:37-41 and Van Keuren *et al.* (1986) *Hum. Genet.* 74:34-40. Alternatively, the presence of a IL-9/IL-2 receptor -like polypeptide in the somatic cell hybrids can be determined by assaying an activity or
25 property of the polypeptide, for example, enzymatic activity, as described in Bordelon-Riser *et al.* (1979) *Somatic Cell Genetics* 5:597-613 and Owerbach *et al.* (1978) *Proc. Natl. Acad. Sci. USA* 75:5640-5644.

Once a sequence has been mapped to a precise chromosomal location, the physical position of the sequence on the chromosome can be correlated with genetic map
30 data. (Such data are found, for example, in V. McKusick, *Mendelian Inheritance in Man*, available on-line through Johns Hopkins University Welch Medical Library). The

relationship between genes and disease, mapped to the same chromosomal region, can then be identified through linkage analysis (co-inheritance of physically adjacent genes), described in, e.g., Egeland *et al.* (1987) *Nature* 325:783-787.

Moreover, differences in the DNA sequences between individuals affected and
5 unaffected with a disease associated with the IL-9/IL-2 receptor-like gene can be determined. If a mutation is observed in some or all of the affected individuals but not in any unaffected individuals, then the mutation is likely to be the causative agent of the particular disease. Comparison of affected and unaffected individuals generally involves first looking for structural alterations in the chromosomes such as deletions or
10 translocations that are visible from chromosome spreads or detectable using PCR based on that DNA sequence. Ultimately, complete sequencing of genes from several individuals can be performed to confirm the presence of a mutation and to distinguish mutations from polymorphisms.

15 2. Tissue Typing

The IL-9/IL-2 receptor-like sequences of the present invention can also be used to identify individuals from minute biological samples. The United States military, for example, is considering the use of restriction fragment length polymorphism (RFLP) for identification of its personnel. In this technique, an individual's genomic DNA is
20 digested with one or more restriction enzymes and probed on a Southern blot to yield unique bands for identification. The sequences of the present invention are useful as additional DNA markers for RFLP (described in U.S. Patent 5,272,057).

Furthermore, the sequences of the present invention can be used to provide an alternative technique for determining the actual base-by-base DNA sequence of selected
25 portions of an individual's genome. Thus, the IL-9/IL-2 receptor-like sequences of the invention can be used to prepare two PCR primers from the 5' and 3' ends of the sequences. These primers can then be used to amplify an individual's DNA and subsequently sequence it.

Panels of corresponding DNA sequences from individuals, prepared in this
30 manner, can provide unique individual identifications, as each individual will have a unique set of such DNA sequences due to allelic differences. The IL-9/IL-2 receptor-like

sequences of the invention uniquely represent portions of the human genome. Allelic variation occurs to some degree in the coding regions of these sequences, and to a greater degree in the noncoding regions. It is estimated that allelic variation between individual humans occurs with a frequency of about once per each 500 bases. Each of the

5 sequences described herein can, to some degree, be used as a standard against which DNA from an individual can be compared for identification purposes. The noncoding sequences of SEQ ID NO:1 or 3 can comfortably provide positive individual identification with a panel of perhaps 10 to 1,000 primers that each yield a noncoding amplified sequence of 100 bases. If a predicted coding sequence, such as that in SEQ ID

10 NO:1 or 3, is used, a more appropriate number of primers for positive individual identification would be 500 to 2,000.

3. Use of Partial IL-9/IL-2 Receptor-like Sequences in Forensic Biology

DNA-based identification techniques can also be used in forensic biology. In this

15 manner, PCR technology can be used to amplify DNA sequences taken from very small biological samples such as tissues, e.g., hair or skin, or body fluids, e.g., blood, saliva, or semen found at a crime scene. The amplified sequence can then be compared to a standard, thereby allowing identification of the origin of the biological sample.

The sequences of the present invention can be used to provide polynucleotide

20 reagents, e.g., PCR primers, targeted to specific loci in the human genome, which can enhance the reliability of DNA-based forensic identifications by, for example, providing another "identification marker" that is unique to a particular individual. As mentioned above, actual base sequence information can be used for identification as an accurate alternative to patterns formed by restriction enzyme generated fragments. Sequences

25 targeted to noncoding regions of SEQ ID NO:1 or 3 are particularly appropriate for this use as greater numbers of polymorphisms occur in the noncoding regions, making it easier to differentiate individuals using this technique. Examples of polynucleotide reagents include the IL-9/IL-2 receptor-like sequences or portions thereof, e.g., fragments derived from the noncoding regions of SEQ ID NO:1 or 3 having

30 a length of at least 20 or 30 bases.

The IL-9/IL-2 receptor-like sequences described herein can further be used to provide polynucleotide reagents, e.g., labeled or labelable probes that can be used in, for example, an *in situ* hybridization technique, to identify a specific tissue. This can be very useful in cases where a forensic pathologist is presented with a tissue of unknown origin.

5 Panels of such IL-9/IL-2 receptor-like probes, can be used to identify tissue by species and/or by organ type.

In a similar fashion, these reagents, e.g., IL-9/IL-2 receptor-like primers or probes can be used to screen tissue culture for contamination (i.e., screen for the presence of a mixture of different types of cells in a culture).

10

C. Predictive Medicine

The present invention also pertains to the field of predictive medicine in which diagnostic assays, prognostic assays, pharmacogenomics, and monitoring clinical trails are used for prognostic (predictive) purposes to thereby treat an individual

15 prophylactically. These applications are described in the subsections below.

1. Diagnostic Assays

One aspect of the present invention relates to diagnostic assays for detecting IL-9/IL-2 receptor-like protein and/or nucleic acid expression as well as IL-9/IL-2 receptor-like activity, in the context of a biological sample. An exemplary method for detecting the presence or absence of IL-9/IL-2 receptor-like proteins in a biological sample involves obtaining a biological sample from a test subject and contacting the biological sample with a compound or an agent capable of detecting IL-9/IL-2 receptor-like protein or nucleic acid (e.g., mRNA, genomic DNA) that encodes IL-9/IL-2 receptor-like protein such that the presence of IL-9/IL-2 receptor-like protein is detected in the biological sample. Results obtained with a biological sample from the test subject may be compared to results obtained with a biological sample from a control subject.

A preferred agent for detecting IL-9/IL-2 receptor-like mRNA or genomic DNA is a labeled nucleic acid probe capable of hybridizing to IL-9/IL-2 receptor-like mRNA or genomic DNA. The nucleic acid probe can be, for example, a full-length IL-9/IL-2 receptor-like nucleic acid, such as the nucleic acid of SEQ ID NO:1 or 3, or a portion

thereof, such as a nucleic acid molecule of at least 15, 30, 50, 100, 250, or 500 nucleotides in length and sufficient to specifically hybridize under stringent conditions to IL-9/IL-2 receptor-like mRNA or genomic DNA. Other suitable probes for use in the diagnostic assays of the invention are described herein.

5 A preferred agent for detecting IL-9/IL-2 receptor-like protein is an antibody capable of binding to IL-9/IL-2 receptor-like protein, preferably an antibody with a detectable label. Antibodies can be polyclonal, or more preferably, monoclonal. An intact antibody, or a fragment thereof (e.g., Fab or F(ab')₂) can be used. The term "labeled", with regard to the probe or antibody, is intended to encompass direct labeling
10 of the probe or antibody by coupling (i.e., physically linking) a detectable substance to the probe or antibody, as well as indirect labeling of the probe or antibody by reactivity with another reagent that is directly labeled. Examples of indirect labeling include detection of a primary antibody using a fluorescently labeled secondary antibody and end-labeling of a DNA probe with biotin such that it can be detected with fluorescently
15 labeled streptavidin.

 The term "biological sample" is intended to include tissues, cells, and biological fluids isolated from a subject, as well as tissues, cells, and fluids present within a subject. That is, the detection method of the invention can be used to detect IL-9/IL-2 receptor-like mRNA, protein, or genomic DNA in a biological sample *in vitro* as well as *in vivo*.
20 For example, *in vitro* techniques for detection of IL-9/IL-2 receptor-like mRNA include Northern hybridizations and *in situ* hybridizations. *In vitro* techniques for detection of IL-9/IL-2 receptor-like protein include enzyme linked immunosorbent assays (ELISAs), Western blots, immunoprecipitations, and immunofluorescence. *In vitro* techniques for detection of IL-9/IL-2 receptor-like genomic DNA include Southern hybridizations.
25 Furthermore, *in vivo* techniques for detection of IL-9/IL-2 receptor-like protein include introducing into a subject a labeled anti-IL-9/IL-2 receptor-like antibody. For example, the antibody can be labeled with a radioactive marker whose presence and location in a subject can be detected by standard imaging techniques.

 In one embodiment, the biological sample contains protein molecules from the
30 test subject. Alternatively, the biological sample can contain mRNA molecules from the test subject or genomic DNA molecules from the test subject. A preferred

biological sample is a peripheral blood leukocyte sample isolated by conventional means from a subject.

The invention also encompasses kits for detecting the presence of IL-9/IL-2 receptor-like proteins in a biological sample (a test sample). Such kits can be used to
5 determine if a subject is suffering from or is at increased risk of developing a disorder associated with aberrant expression of IL-9/IL-2 receptor-like protein (e.g., an immunological disorder). For example, the kit can comprise a labeled compound or agent capable of detecting IL-9/IL-2 receptor-like protein or mRNA in a biological
10 sample and means for determining the amount of an IL-9/IL-2 receptor-like protein in the sample (e.g., an anti-IL-9/IL-2 receptor-like antibody or an oligonucleotide probe that binds to DNA encoding an IL-9/IL-2 receptor-like protein, e.g., SEQ ID NO:1 or 3). Kits can also include instructions for observing that the tested subject is suffering from or is at risk of developing a disorder associated with aberrant expression of IL-9/IL-2 receptor-like sequences if the amount of IL-9/IL-2 receptor-like protein or mRNA is above or
15 below a normal level.

For antibody-based kits, the kit can comprise, for example: (1) a first antibody (e.g., attached to a solid support) that binds to IL-9/IL-2 receptor-like protein; and, optionally, (2) a second, different antibody that binds to IL-9/IL-2 receptor-like protein or the first antibody and is conjugated to a detectable agent. For oligonucleotide-based kits,
20 the kit can comprise, for example: (1) an oligonucleotide, e.g., a detectably labeled oligonucleotide, that hybridizes to an IL-9/IL-2 receptor-like nucleic acid sequence or (2) a pair of primers useful for amplifying an IL-9/IL-2 receptor-like nucleic acid molecule.

The kit can also comprise, e.g., a buffering agent, a preservative, or a protein stabilizing agent. The kit can also comprise components necessary for detecting the
25 detectable agent (e.g., an enzyme or a substrate). The kit can also contain a control sample or a series of control samples that can be assayed and compared to the test sample contained. Each component of the kit is usually enclosed within an individual container, and all of the various containers are within a single package along with instructions for observing whether the tested subject is suffering from or is at risk of developing a
30 disorder associated with aberrant expression of IL-9/IL-2 receptor-like proteins.

2. Other Diagnostic Assays

In another aspect, the invention features a method of analyzing a plurality of capture probes. The method can be used, e.g., to analyze gene expression. The method includes: providing a two dimensional array having a plurality of addresses, each address of the plurality being positionally distinguishable from each other address of the plurality, and each address of the plurality having a unique capture probe, e.g., a nucleic acid or peptide sequence; contacting the array with an IL-9/IL-2 receptor-like nucleic acid, preferably purified, polypeptide, preferably purified, or antibody, and thereby evaluating the plurality of capture probes. Binding, e.g., in the case of a nucleic acid, hybridization, with a capture probe at an address of the plurality, is detected, e.g., by signal generated from a label attached to the IL-9/IL-2 receptor-like nucleic acid, polypeptide, or antibody. The capture probes can be a set of nucleic acids from a selected sample, e.g., a sample of nucleic acids derived from a control or non-stimulated tissue or cell.

The method can include contacting the IL-9/IL-2 receptor-like nucleic acid, polypeptide, or antibody with a first array having a plurality of capture probes and a second array having a different plurality of capture probes. The results of each hybridization can be compared, e.g., to analyze differences in expression between a first and second sample. The first plurality of capture probes can be from a control sample, e.g., a wild type, normal, or non-diseased, non-stimulated, sample, e.g., a biological fluid, tissue, or cell sample. The second plurality of capture probes can be from an experimental sample, e.g., a mutant type, at risk, disease-state or disorder-state, or stimulated, sample, e.g., a biological fluid, tissue, or cell sample.

The plurality of capture probes can be a plurality of nucleic acid probes each of which specifically hybridizes, with an allele of an IL-9/IL-2 receptor-like sequence of the invention. Such methods can be used to diagnose a subject, e.g., to evaluate risk for a disease or disorder, to evaluate suitability of a selected treatment for a subject, to evaluate whether a subject has a disease or disorder. Thus, for example, the h16445 sequence set forth in SEQ ID NO:1 encodes an IL-9/IL-2 receptor-like polypeptide that is associated with immune function, thus it is useful for evaluating immune disorders.

The method can be used to detect single nucleotide polymorphisms (SNPs), as described below.

In another aspect, the invention features a method of analyzing a plurality of probes. The method is useful, e.g., for analyzing gene expression. The method includes:

5 providing a two dimensional array having a plurality of addresses, each address of the plurality being positionally distinguishable from each other address of the plurality having a unique capture probe, e.g., wherein the capture probes are from a cell or subject which express an IL-9/IL-2 receptor-like polypeptide of the invention or from a cell or subject in which an IL-9/IL-2 receptor-like-mediated response has been elicited, e.g., by

10 contact of the cell with an IL-9/IL-2 receptor-like nucleic acid or protein of the invention, or administration to the cell or subject an IL-9/IL-2 receptor-like nucleic acid or protein of the invention; contacting the array with one or more inquiry probes, wherein an inquiry probe can be a nucleic acid, polypeptide, or antibody (which is preferably other than an IL-9/IL-2 receptor-like nucleic acid, polypeptide, or antibody of the invention);

15 providing a two dimensional array having a plurality of addresses, each address of the plurality being positionally distinguishable from each other address of the plurality, and each address of the plurality having a unique capture probe, e.g., wherein the capture probes are from a cell or subject which does not express an IL-9/IL-2 receptor-like sequence of the invention (or does not express as highly as in the case of the IL-9/IL-2

20 receptor-like positive plurality of capture probes) or from a cell or subject in which an IL-9/IL-2 receptor-like-mediated response has not been elicited (or has been elicited to a lesser extent than in the first sample); contacting the array with one or more inquiry probes (which is preferably other than an IL-9/IL-2 receptor-like nucleic acid, polypeptide, or antibody of the invention), and thereby evaluating the plurality of capture

25 probes. Binding, e.g., in the case of a nucleic acid, hybridization, with a capture probe at an address of the plurality, is detected, e.g., by signal generated from a label attached to the nucleic acid, polypeptide, or antibody.

In another aspect, the invention features a method of analyzing an IL-9/IL-2 receptor-like sequence of the invention, e.g., analyzing structure, function, or relatedness

30 to other nucleic acid or amino acid sequences. The method includes: providing an IL-9/IL-2 receptor-like nucleic acid or amino acid sequence, e.g., the h16445 sequence set

forth in SEQ ID NO:1 or a portion thereof; comparing the IL-9/IL-2 receptor-like sequence with one or more preferably a plurality of sequences from a collection of sequences, e.g., a nucleic acid or protein sequence database; to thereby analyze the IL-9/IL-2 receptor-like sequence of the invention.

5 The method can include evaluating the sequence identity between an IL-9/IL-2 receptor-like sequence of the invention, e.g., the h16445 sequence, and a database sequence. The method can be performed by accessing the database at a second site, e.g., over the internet.

10 In another aspect, the invention features, a set of oligonucleotides, useful, e.g., for identifying SNP's, or identifying specific alleles of an IL-9/IL-2 receptor-like sequence of the invention, e.g., the h16445 sequence. The set includes a plurality of oligonucleotides, each of which has a different nucleotide at an interrogation position, e.g., an SNP or the site of a mutation. In a preferred embodiment, the oligonucleotides of the plurality identical in sequence with one another (except for differences in length).

15 The oligonucleotides can be provided with differential labels, such that an oligonucleotides which hybridizes to one allele provides a signal that is distinguishable from an oligonucleotides which hybridizes to a second allele.

3. Prognostic Assays

20 The methods described herein can furthermore be utilized as diagnostic or prognostic assays to identify subjects having or at risk of developing a disease or disorder associated with IL-9/IL-2 receptor-like protein, IL-9/IL-2 receptor-like nucleic acid expression, or IL-9/IL-2 receptor-like activity. Prognostic assays can be used for prognostic or predictive purposes to thereby prophylactically treat an individual prior to
25 the onset of a disorder characterized by or associated with IL-9/IL-2 receptor-like protein, IL-9/IL-2 receptor-like nucleic acid expression, or IL-9/IL-2 receptor-like activity.

30 Thus, the present invention provides a method in which a test sample is obtained from a subject, and IL-9/IL-2 receptor-like protein or nucleic acid (e.g., mRNA, genomic DNA) is detected, wherein the presence of IL-9/IL-2 receptor-like protein or nucleic acid is diagnostic for a subject having or at risk of developing a disease or disorder associated with aberrant IL-9/IL-2 receptor-like expression or activity. As used herein, a "test

sample" refers to a biological sample obtained from a subject of interest. For example, a test sample can be a biological fluid (e.g., serum), cell sample, or tissue.

Furthermore, using the prognostic assays described herein, the present invention provides methods for determining whether a subject can be administered a specific agent (e.g., an agonist, antagonist, peptidomimetic, protein, peptide, nucleic acid, small molecule, or other drug candidate) or class of agents (e.g., agents of a type that decrease IL-9/IL-2 receptor-like activity) to effectively treat a disease or disorder associated with aberrant IL-9/IL-2 receptor-like expression or activity. In this manner, a test sample is obtained and IL-9/IL-2 receptor-like protein or nucleic acid is detected. The presence of IL-9/IL-2 receptor-like protein or nucleic acid is diagnostic for a subject that can be administered the agent to treat a disorder associated with aberrant IL-9/IL-2 receptor-like expression or activity.

The methods of the invention can also be used to detect genetic lesions or mutations in an IL-9/IL-2 receptor-like gene, thereby determining if a subject with the lesioned gene is at risk for a disorder characterized by aberrant cell proliferation and/or differentiation. In preferred embodiments, the methods include detecting, in a sample of cells from the subject, the presence or absence of a genetic lesion or mutation characterized by at least one of an alteration affecting the integrity of a gene encoding an IL-9/IL-2 receptor-like-protein, or the misexpression of the IL-9/IL-2 receptor-like gene. For example, such genetic lesions or mutations can be detected by ascertaining the existence of at least one of: (1) a deletion of one or more nucleotides from an IL-9/IL-2 receptor-like gene; (2) an addition of one or more nucleotides to an IL-9/IL-2 receptor-like gene; (3) a substitution of one or more nucleotides of an IL-9/IL-2 receptor-like gene; (4) a chromosomal rearrangement of an IL-9/IL-2 receptor-like gene; (5) an alteration in the level of a messenger RNA transcript of an IL-9/IL-2 receptor-like gene; (6) an aberrant modification of an IL-9/IL-2 receptor-like gene, such as of the methylation pattern of the genomic DNA; (7) the presence of a non-wild-type splicing pattern of a messenger RNA transcript of an IL-9/IL-2 receptor-like gene; (8) a non-wild-type level of an IL-9/IL-2 receptor-like-protein; (9) an allelic loss of an IL-9/IL-2 receptor-like gene; and (10) an inappropriate post-translational modification of an IL-9/IL-2 receptor-like-protein. As described herein, there are a large number of assay

techniques known in the art that can be used for detecting lesions in an IL-9/IL-2 receptor-like gene. Any cell type or tissue, preferably peripheral blood leukocytes, in which IL-9/IL-2 receptor-like proteins are expressed may be utilized in the prognostic assays described herein.

5 In certain embodiments, detection of the lesion involves the use of a probe/primer in a polymerase chain reaction (PCR) (*see, e.g.,* U.S. Patent Nos. 4,683,195 and 4,683,202), such as anchor PCR or RACE PCR, or, alternatively, in a ligation chain reaction (LCR) (*see, e.g.,* Landegran *et al.* (1988) *Science* 241:1077-1080; and Nakazawa *et al.* (1994) *Proc. Natl. Acad. Sci. USA* 91:360-364), the latter of which can be
10 particularly useful for detecting point mutations in the IL-9/IL-2 receptor-like-gene (*see, e.g.,* Abravaya *et al.* (1995) *Nucleic Acids Res.* 23:675-682). It is anticipated that PCR and/or LCR may be desirable to use as a preliminary amplification step in conjunction with any of the techniques used for detecting mutations described herein.

Alternative amplification methods include self sustained sequence replication
15 (Guatelli *et al.* (1990) *Proc. Natl. Acad. Sci. USA* 87:1874-1878), transcriptional amplification system (Kwoh *et al.* (1989) *Proc. Natl. Acad. Sci. USA* 86:1173-1177), Q-Beta Replicase (Lizardi *et al.* (1988) *Bio/Technology* 6:1197), or any other nucleic acid amplification method, followed by the detection of the amplified molecules using techniques well known to those of skill in the art. These detection schemes are especially
20 useful for the detection of nucleic acid molecules if such molecules are present in very low numbers.

In an alternative embodiment, mutations in an IL-9/IL-2 receptor-like gene from a sample cell can be identified by alterations in restriction enzyme cleavage patterns of isolated test sample and control DNA digested with one or more restriction
25 endonucleases. Moreover, the use of sequence specific ribozymes (*see, e.g.,* U.S. Patent No. 5,498,531) can be used to score for the presence of specific mutations by development or loss of a ribozyme cleavage site.

In other embodiments, genetic mutations in an IL-9/IL-2 receptor-like molecule can be identified by hybridizing a sample and control nucleic acids, *e.g.,* DNA or RNA,
30 to high density arrays containing hundreds or thousands of oligonucleotides probes (Cronin *et al.* (1996) *Human Mutation* 7:244-255; Kozal *et al.* (1996) *Nature Medicine*

2:753-759). In yet another embodiment, any of a variety of sequencing reactions known in the art can be used to directly sequence the IL-9/IL-2 receptor-like gene and detect mutations by comparing the sequence of the sample IL-9/IL-2 receptor-like gene with the corresponding wild-type (control) sequence. Examples of sequencing reactions include those based on techniques developed by Maxim and Gilbert ((1977) *Proc. Natl. Acad. Sci. USA* 74:560) or Sanger ((1977) *Proc. Natl. Acad. Sci. USA* 74:5463). It is also contemplated that any of a variety of automated sequencing procedures can be utilized when performing the diagnostic assays ((1995) *Bio/Techniques* 19:448), including sequencing by mass spectrometry (*see, e.g.,* PCT Publication No. WO 94/16101; Cohen *et al.* (1996) *Adv. Chromatogr.* 36:127-162; and Griffin *et al.* (1993) *Appl. Biochem. Biotechnol.* 38:147-159).

Other methods for detecting mutations in the IL-9/IL-2 receptor-like gene include methods in which protection from cleavage agents is used to detect mismatched bases in RNA/RNA or RNA/DNA heteroduplexes (Myers *et al.* (1985) *Science* 230:1242). *See, also* Cotton *et al.* (1988) *Proc. Natl. Acad. Sci. USA* 85:4397; Saleeba *et al.* (1992) *Methods Enzymol.* 217:286-295. In a preferred embodiment, the control DNA or RNA can be labeled for detection.

In still another embodiment, the mismatch cleavage reaction employs one or more "DNA mismatch repair" enzymes that recognize mismatched base pairs in double-stranded DNA in defined systems for detecting and mapping point mutations in IL-9/IL-2 receptor-like cDNAs obtained from samples of cells. *See, e.g.,* Hsu *et al.* (1994) *Carcinogenesis* 15:1657-1662. According to an exemplary embodiment, a probe based on an IL-9/IL-2 receptor-like sequence, *e.g.,* a wild-type IL-9/IL-2 receptor-like sequence, is hybridized to a cDNA or other DNA product from a test cell(s). The duplex is treated with a DNA mismatch repair enzyme, and the cleavage products, if any, can be detected from electrophoresis protocols or the like. *See, e.g.,* U.S. Patent No. 5,459,039.

In other embodiments, alterations in electrophoretic mobility will be used to identify mutations in IL-9/IL-2 receptor-like genes. For example, single-strand conformation polymorphism (SSCP) may be used to detect differences in electrophoretic mobility between mutant and wild-type nucleic acids (Orita *et al.* (1989) *Proc. Natl. Acad. Sci. USA* 86:2766; *see also* Cotton (1993) *Mutat. Res.* 285:125-144; Hayashi

(1992) *Genet. Anal. Tech. Appl.* 9:73-79). The sensitivity of the assay may be enhanced by using RNA (rather than DNA), in which the secondary structure is more sensitive to a change in sequence. In a preferred embodiment, the subject method utilizes heteroduplex analysis to separate double-stranded heteroduplex molecules on the basis of changes in electrophoretic mobility (Keen *et al.* (1991) *Trends Genet.* 7:5).

In yet another embodiment, the movement of mutant or wild-type fragments in polyacrylamide gels containing a gradient of denaturant is assayed using denaturing gradient gel electrophoresis (DGGE) (Myers *et al.* (1985) *Nature* 313:495). When DGGE is used as the method of analysis, DNA will be modified to insure that it does not completely denature, for example by adding a GC clamp of approximately 40 bp of high-melting GC-rich DNA by PCR. In a further embodiment, a temperature gradient is used in place of a denaturing gradient to identify differences in the mobility of control and sample DNA (Rosenbaum and Reissner (1987) *Biophys. Chem.* 265:12753).

Examples of other techniques for detecting point mutations include, but are not limited to, selective oligonucleotide hybridization, selective amplification, or selective primer extension. For example, oligonucleotide primers may be prepared in which the known mutation is placed centrally and then hybridized to target DNA under conditions that permit hybridization only if a perfect match is found (Saiki *et al.* (1986) *Nature* 324:163); Saiki *et al.* (1989) *Proc. Natl. Acad. Sci. USA* 86:6230). Such allele-specific oligonucleotides are hybridized to PCR-amplified target DNA or a number of different mutations when the oligonucleotides are attached to the hybridizing membrane and hybridized with labeled target DNA.

Alternatively, allele-specific amplification technology, which depends on selective PCR amplification, may be used in conjunction with the instant invention. Oligonucleotides used as primers for specific amplification may carry the mutation of interest in the center of the molecule so that amplification depends on differential hybridization (Gibbs *et al.* (1989) *Nucleic Acids Res.* 17:2437-2448) or at the extreme 3' end of one primer where, under appropriate conditions, mismatch can prevent or reduce polymerase extension (Prossner (1993) *Tibtech* 11:238). In addition, it may be desirable to introduce a novel restriction site in the region of the mutation to create cleavage-based

detection (Gasparini *et al.* (1992) *Mol. Cell Probes* 6:1). It is anticipated that in certain embodiments amplification may also be performed using Taq ligase for amplification (Barany (1991) *Proc. Natl. Acad. Sci. USA* 88:189). In such cases, ligation will occur only if there is a perfect match at the 3' end of the 5' sequence making it possible to
5 detect the presence of a known mutation at a specific site by looking for the presence or absence of amplification.

The methods described herein may be performed, for example, by utilizing prepackaged diagnostic kits comprising at least one probe nucleic acid or antibody reagent described herein, which may be conveniently used, e.g., in clinical settings to
10 diagnosed patients exhibiting symptoms or family history of a disease or illness involving an IL-9/IL-2 receptor-like gene.

4. Pharmacogenomics

Agents, or modulators that have a stimulatory or inhibitory effect on IL-9/IL-2
15 receptor-like activity (e.g., IL-9/IL-2 receptor-like gene expression) as identified by a screening assay described herein, can be administered to individuals to treat (prophylactically or therapeutically) disorders associated with aberrant IL-9/IL-2 receptor-like activity as well as to modulate the phenotype of an immune response. In conjunction with such treatment, the pharmacogenomics (i.e., the study of the
20 relationship between an individual's genotype and that individual's response to a foreign compound or drug) of the individual may be considered. Differences in metabolism of therapeutics can lead to severe toxicity or therapeutic failure by altering the relation between dose and blood concentration of the pharmacologically active drug. Thus, the pharmacogenomics of the individual permits the selection of effective agents (e.g., drugs)
25 for prophylactic or therapeutic treatments based on a consideration of the individual's genotype. Such pharmacogenomics can further be used to determine appropriate dosages and therapeutic regimens. Accordingly, the activity of IL-9/IL-2 receptor-like protein, expression of IL-9/IL-2 receptor-like nucleic acid, or mutation content of IL-9/IL-2 receptor-like genes in an individual can be determined to thereby select appropriate
30 agent(s) for therapeutic or prophylactic treatment of the individual.

Pharmacogenomics deals with clinically significant hereditary variations in the response to drugs due to altered drug disposition and abnormal action in affected persons. See, e.g., Linder (1997) *Clin. Chem.* 43(2):254-266. In general, two types of pharmacogenetic conditions can be differentiated. Genetic conditions transmitted as a single factor altering the way drugs act on the body are referred to as "altered drug action." Genetic conditions transmitted as single factors altering the way the body acts on drugs are referred to as "altered drug metabolism". These pharmacogenetic conditions can occur either as rare defects or as polymorphisms. For example, glucose-6-phosphate dehydrogenase deficiency (G6PD) is a common inherited enzymopathy in which the main clinical complication is haemolysis after ingestion of oxidant drugs (antimalarials, sulfonamides, analgesics, nitrofurans) and consumption of fava beans.

One pharmacogenomics approach to identifying genes that predict drug response, known as "a genome-wide association", relies primarily on a high-resolution map of the human genome consisting of already known gene-related markers (e.g., a "bi-allelic" gene marker map which consists of 60,000-100,000 polymorphic or variable sites on the human genome, each of which has two variants.) Such a high-resolution genetic map can be compared to a map of the genome of each of a statistically significant number of patients taking part in a Phase II/III drug trial to identify markers associated with a particular observed drug response or side effect. Alternatively, such a high resolution map can be generated from a combination of some ten-million known single nucleotide polymorphisms (SNPs) in the human genome. As used herein, an "SNP" is a common alteration that occurs in a single nucleotide base in a stretch of DNA. For example, a SNP may occur once per every 1000 bases of DNA. A SNP may be involved in a disease process, however, the vast majority may not be disease-associated. Given a genetic map based on the occurrence of such SNPs, individuals can be grouped into genetic categories depending on a particular pattern of SNPs in their individual genome. In such a manner, treatment regimens can be tailored to groups of genetically similar individuals, taking into account traits that may be common among such genetically similar individuals.

Alternatively, a method termed the "candidate gene approach", can be utilized to identify genes that predict drug response. According to this method, if a gene that encodes a drug's target is known (e.g., an IL-9/IL-2 receptor-like protein of the present

invention), all common variants of that gene can be fairly easily identified in the population and it can be determined if having one version of the gene versus another is associated with a particular drug response.

Alternatively, a method termed the "gene expression profiling", can be utilized to
5 identify genes that predict drug response. For example, the gene expression of an animal dosed with a drug (e.g., an IL-9/IL-2 receptor-like molecule or IL-9/IL-2 receptor-like modulator of the present invention) can give an indication whether gene pathways related to toxicity have been turned on.

Information generated from more than one of the above pharmacogenomics
10 approaches can be used to determine appropriate dosage and treatment regimens for prophylactic or therapeutic treatment of an individual. This knowledge, when applied to dosing or drug selection, can avoid adverse reactions or therapeutic failure and thus enhance therapeutic or prophylactic efficiency when treating a subject with an IL-9/IL-2 receptor-like molecule or IL-9/IL-2 receptor-like modulator of the invention, such as a
15 modulator identified by one of the exemplary screening assays described herein.

The present invention further provides methods for identifying new agents, or combinations, that are based on identifying agents that modulate the activity of one or more of the gene products encoded by one or more of the IL-9/IL-2 receptor-like genes of the present invention, wherein these products may be associated with resistance of the
20 cells to a therapeutic agent. Specifically, the activity of the proteins encoded by the IL-9/IL-2 receptor-like genes of the present invention can be used as a basis for identifying agents for overcoming agent resistance. By blocking the activity of one or more of the resistance proteins, target cells, e.g., B-cells and colon cells, will become sensitive to treatment with an agent that the unmodified target cells were resistant to.

Monitoring the influence of agents (e.g., drugs) on the expression or activity of an
25 IL-9/IL-2 receptor-like protein can be applied in clinical trials. For example, the effectiveness of an agent determined by a screening assay as described herein to increase IL-9/IL-2 receptor-like gene expression, protein levels, or upregulate IL-9/IL-2 receptor-like activity, can be monitored in clinical trials of subjects exhibiting decreased IL-9/IL-2
30 receptor-like gene expression, protein levels, or downregulated IL-9/IL-2 receptor-like activity. Alternatively, the effectiveness of an agent determined by a screening assay to

decrease IL-9/IL-2 receptor-like gene expression, protein levels, or downregulate IL-9/IL-2 receptor-like activity, can be monitored in clinical trials of subjects exhibiting increased IL-9/IL-2 receptor-like gene expression, protein levels, or upregulated IL-9/IL-2 receptor-like activity. In such clinical trials, the expression or activity of an IL-9/IL-2 receptor-like gene, and preferably, other genes that have been implicated in, for example, an IL-9/IL-2 receptor-like-associated disorder can be used as a "read out" or markers of the phenotype of a particular cell.

As an illustrative embodiment, the activity of drug metabolizing enzymes is a major determinant of both the intensity and duration of drug action. The discovery of genetic polymorphisms of drug metabolizing enzymes (e.g., N-acetyltransferase 2 (NAT 2) and cytochrome P450 enzymes CYP2D6 and CYP2C19) has provided an explanation as to why some patients do not obtain the expected drug effects or show exaggerated drug response and serious toxicity after taking the standard and safe dose of a drug. These polymorphisms are expressed in two phenotypes in the population, the extensive metabolizer (EM) and poor metabolizer (PM). The prevalence of PM is different among different populations. For example, the gene coding for CYP2D6 is highly polymorphic and several mutations have been identified in PM, which all lead to the absence of functional CYP2D6. Poor metabolizers of CYP2D6 and CYP2C19 quite frequently experience exaggerated drug response and side effects when they receive standard doses. If a metabolite is the active therapeutic moiety, a PM will show no therapeutic response, as demonstrated for the analgesic effect of codeine mediated by its CYP2D6-formed metabolite morphine. The other extreme are the so called ultra-rapid metabolizers who do not respond to standard doses. Recently, the molecular basis of ultra-rapid metabolism has been identified to be due to CYP2D6 gene amplification.

Thus, the activity of IL-9/IL-2 receptor-like protein, expression of IL-9/IL-2 receptor-like nucleic acid, or mutation content of IL-9/IL-2 receptor-like genes in an individual can be determined to thereby select appropriate agent(s) for therapeutic or prophylactic treatment of the individual. In addition, pharmacogenetic studies can be used to apply genotyping of polymorphic alleles encoding drug-metabolizing enzymes to the identification of an individual's drug responsiveness phenotype. This knowledge, when applied to dosing or drug selection, can avoid adverse reactions or therapeutic

failure and thus enhance therapeutic or prophylactic efficiency when treating a subject with an IL-9/IL-2 receptor-like modulator, such as a modulator identified by one of the exemplary screening assays described herein.

5 5. Monitoring of Effects During Clinical Trials

Monitoring the influence of agents (e.g., drugs, compounds) on the expression or activity of IL-9/IL-2 receptor-like genes (e.g., the ability to modulate aberrant cell proliferation and/or differentiation) can be applied not only in basic drug screening but also in clinical trials. For example, the effectiveness of an agent, as determined by a screening assay as described herein, to increase or decrease IL-9/IL-2 receptor-like gene expression, protein levels, or protein activity, can be monitored in clinical trials of subjects exhibiting decreased or increased IL-9/IL-2 receptor-like gene expression, protein levels, or protein activity. In such clinical trials, IL-9/IL-2 receptor-like expression or activity and preferably that of other genes that have been implicated in for example, a cellular proliferation disorder, can be used as a marker of the immune responsiveness of a particular cell.

For example, and not by way of limitation, genes that are modulated in cells by treatment with an agent (e.g., compound, drug, or small molecule) that modulates IL-9/IL-2 receptor-like activity (e.g., as identified in a screening assay described herein) can be identified. Thus, to study the effect of agents on cellular proliferation disorders, for example, in a clinical trial, cells can be isolated and RNA prepared and analyzed for the levels of expression of IL-9/IL-2 receptor-like genes and other genes implicated in the disorder. The levels of gene expression (i.e., a gene expression pattern) can be quantified by Northern blot analysis or RT-PCR, as described herein, or alternatively by measuring the amount of protein produced, by one of the methods as described herein, or by measuring the levels of activity of IL-9/IL-2 receptor-like genes or other genes. In this way, the gene expression pattern can serve as a marker, indicative of the physiological response of the cells to the agent. Accordingly, this response state may be determined before, and at various points during, treatment of the individual with the agent.

In a preferred embodiment, the present invention provides a method for monitoring the effectiveness of treatment of a subject with an agent (e.g., an agonist,

antagonist, peptidomimetic, protein, peptide, nucleic acid, small molecule, or other drug candidate identified by the screening assays described herein) comprising the steps of (1) obtaining a preadministration sample from a subject prior to administration of the agent; (2) detecting the level of expression of an IL-9/IL-2 receptor-like protein, mRNA, or genomic DNA in the preadministration sample; (3) obtaining one or more postadministration samples from the subject; (4) detecting the level of expression or activity of the IL-9/IL-2 receptor-like protein, mRNA, or genomic DNA in the postadministration samples; (5) comparing the level of expression or activity of the IL-9/IL-2 receptor-like protein, mRNA, or genomic DNA in the preadministration sample with the IL-9/IL-2 receptor-like protein, mRNA, or genomic DNA in the postadministration sample or samples; and (vi) altering the administration of the agent to the subject accordingly to bring about the desired effect, i.e., for example, an increase or a decrease in the expression or activity of an IL-9/IL-2 receptor-like protein.

C. Methods of Treatment

The present invention provides for both prophylactic and therapeutic methods of treating a subject at risk of (or susceptible to) a disorder or having a disorder associated with aberrant IL-9/IL-2 receptor-like expression or activity. Additionally, the compositions of the invention find use in modulating the T-lymphocyte response. Thus, therapies for immune and respiratory disorders are encompassed herein.

1. Prophylactic Methods

In one aspect, the invention provides a method for preventing in a subject a disease or condition associated with an aberrant IL-9/IL-2 receptor-like expression or activity by administering to the subject an agent that modulates IL-9/IL-2 receptor-like expression or at least one IL-9/IL-2 receptor-like gene activity. Subjects at risk for a disease that is caused, or contributed to, by aberrant IL-9/IL-2 receptor-like expression or activity can be identified by, for example, any or a combination of diagnostic or prognostic assays as described herein. Administration of a prophylactic agent can occur prior to the manifestation of symptoms characteristic of the IL-9/IL-2 receptor-like aberrancy, such that a disease or disorder is prevented or, alternatively, delayed in its

progression. Depending on the type of IL-9/IL-2 receptor-like aberrancy, for example, an IL-9/IL-2 receptor-like agonist or IL-9/IL-2 receptor-like antagonist agent can be used for treating the subject. The appropriate agent can be determined based on screening assays described herein.

5

2. Therapeutic Methods

Another aspect of the invention pertains to methods of modulating IL-9/IL-2 receptor-like expression or activity for therapeutic purposes. The modulatory method of the invention involves contacting a cell with an agent that modulates one or more of the activities of IL-9/IL-2 receptor-like protein activity associated with the cell. An agent that modulates IL-9/IL-2 receptor-like protein activity can be an agent as described herein, such as a nucleic acid or a protein, a naturally-occurring cognate ligand of an IL-9/IL-2 receptor-like protein, a peptide, an IL-9/IL-2 receptor-like peptidomimetic, or other small molecule. In one embodiment, the agent stimulates one or more of the biological activities of IL-9/IL-2 receptor-like protein. Examples of such stimulatory agents include active IL-9/IL-2 receptor-like protein and a nucleic acid molecule encoding an IL-9/IL-2 receptor-like protein that has been introduced into the cell. In another embodiment, the agent inhibits one or more of the biological activities of IL-9/IL-2 receptor-like protein. Examples of such inhibitory agents include antisense IL-9/IL-2 receptor-like nucleic acid molecules and anti-IL-9/IL-2 receptor-like antibodies.

These modulatory methods can be performed *in vitro* (e.g., by culturing the cell with the agent) or, alternatively, *in vivo* (e.g. by administering the agent to a subject). As such, the present invention provides methods of treating an individual afflicted with a disease or disorder characterized by aberrant expression or activity of an IL-9/IL-2 receptor-like protein or nucleic acid molecule. In one embodiment, the method involves administering an agent (e.g., an agent identified by a screening assay described herein), or a combination of agents, that modulates (e.g., upregulates or downregulates) IL-9/IL-2 receptor-like expression or activity. In another embodiment, the method involves administering an IL-9/IL-2 receptor-like protein or nucleic acid molecule as therapy to compensate for reduced or aberrant IL-9/IL-2 receptor-like expression or activity.

Stimulation of IL-9/IL-2 receptor-like activity is desirable in situations in which an IL-9/IL-2 receptor-like protein is abnormally downregulated and/or in which increased IL-9/IL-2 receptor-like activity is likely to have a beneficial effect. Conversely, inhibition of IL-9/IL-2 receptor-like activity is desirable in situations in which IL-9/IL-2 receptor-like activity is abnormally upregulated and/or in which decreased IL-9/IL-2 receptor-like activity is likely to have a beneficial effect.

This invention is further illustrated by the following examples, which should not be construed as limiting.

EXPERIMENTAL

Example 1: Isolation of h16445 and m16445

Peripheral blood lymphocytes purified from heparinized blood of 22 normal donors depleted of B cells using anti-CD19 beads (Miltenyi Biotec, Inc.) were combined in RPMI media containing 10% fetal bovine serum and incubated at 37°C for 4, 14, and 24 hours. Harvested cells were pooled for RNA purification. Poly-A⁺ RNA was converted to cDNA using oligo dT primers and reverse transcriptase and cloned into pMET to generate a cDNA library. The average insert size from this library was 1500 nucleotide base pairs. EST sequencing was performed on this library, and greater than 10,000 sequences were subjected to database analysis together with other proprietary sequences.

From this analysis, two distinct sequences, jthLa064e09t1 and jthLa065b03t1, were combined into a single contiguous sequence and identified by BLASTX sequence analysis to be similar to known cytokine receptors. No other sequences were included in this contig. The highest blast hit at that time was SP Accession No. Q01114 (SEQ ID NO:8), which codes for murine interleukin-9 receptor (IL-9R).

Clones corresponding to this single contiguous sequence were retrieved and subjected to full sequence determination and reanalysis. This new sequence, while still similar to the cytokine receptor family, did not appear to contain the entire open reading frame for the protein. Primers designed from the 5' end of the sequence were used to PCR-amplify sequences using RACE technology. These clones were subjected to

sequence determination and analysis, which revealed a more extensive reading frame with further similarity to known cytokine receptors. Upon further analysis, the clone h16445 was identified.

The identified clone h16445 encodes a transcript of approximately 2.3 Kb
5 (corresponding cDNA set forth in SEQ ID NO:1). The open reading frame (nt 349-1965) of this transcript encodes a predicted 538 amino acid protein (SEQ ID NO:2) having a molecular weight of approximately 59.1 kDa. A search of the nucleotide and protein databases revealed that h16445 encodes a precursor polypeptide that shares similarity with several cytokine receptor proteins. An alignment of the protein sequences having
10 highest similarity to the h16445 precursor polypeptide is shown in Figure 1. The alignment was generated using the Clustal method with PAM250 residue weight table and sequence identities were determined by FASTA (Pearson and Lipman (1988) *Proc. Natl. Acad. Sci. USA* 85:2444-2448).

The h16445 protein displays similarity to the human IL-2 receptor beta chain
15 (approximately 36.9% over a 130 amino acid overlap; SEQ ID NO:5; SP Accession Number P14784; Hatakeyama *et al.* (1989) *Science* 244(4904):551-556). It also displays similarity to the murine IL-2 receptor beta chain (approximately 32.7% identity over a 110 amino acid overlap; SEQ ID NO:6; SP Accession Number P16297; Kono *et al.* (1990) *Proc. Natl. Acad. Sci. USA* 87(5):1806-1810); the human IL-9 receptor
20 (approximately 29.7% identity over a 158 amino acid overlap; SEQ ID NO:7; SP Accession Number Q01113; Renauld *et al.* (1992) *Proc. Natl. Acad. Sci. USA* 89(12):5690-5694; Chang *et al.* (1994) *Blood* 83(11):3199-3205; Kermouni *et al.* (1995) *Genomics* 29(2):371-382); (approximately 28.3% identity over a 166 amino acid overlap) to the murine IL-9 receptor (SEQ ID NO:8; SP Accession Number Q01114; Renauld *et al.* (1992) *Proc. Natl. Acad. Sci. USA* 89(12):5690-5694) (see Figure 1).
25

Using a similar database mining strategy, clone m16445 was identified from a three-week-old murine LTBM (long-term bone marrow cell) library made by stimulating the cells with heat-inactivated yeast hyphae at a hyphae:cell ratio of 2.3:1. The identified clone m16445 encodes a transcript of approximately 2.5 Kb
30 (corresponding cDNA set forth in SEQ ID NO:3). The open reading frame (nt 391-1976) of this transcript encodes a predicted 529 amino acid protein (SEQ ID NO:4) having a

molecular weight of approximately 58.3 kDa. This polypeptide represents a protein sequence encoded by the murine orthologue of the h16445 gene. An alignment of the m16445 protein with the h16445 protein reveals that these polypeptides share approximately 64.4% identity as determined by pairwise alignment (see Needleman and Wunsch (1970) *J. Mol. Biol.* 48:444).

Example 2: mRNA Expression and *In Situ* Expression of Clone h16445

A Northern blot analysis of h16445 revealed expression in a number of tissues, including the following, in order of highest to lowest expression: skeletal muscle, lymph node, thymus, spleen, brain, liver fibrosis, fetal liver, lung, and liver, with skeletal muscle exhibiting an expression level about 20-fold higher than that exhibited by lymph node.

Expression of h16445 was measured by TaqMan[®] quantitative PCR (Perkin Elmer Applied Biosystems) in cDNA prepared from the following normal human tissues: lymph node, spleen, thymus, brain, lung, skeletal muscle, fetal liver, and normal and fibrotic liver; the following primary cells: resting and phytohemagglutinin (PHA) activated peripheral blood mononuclear cells (PBMC); resting and PHA activated CD3⁺ cells, CD4⁺ and CD8⁺ T cells; Th1 and Th2 cells stimulated for six or 48 hours with anti-CD3 antibody; resting and lipopolysaccharide (LPS) activated CD19⁺ B cells; CD34⁺ cells from mobilized peripheral blood (mPB CD34⁺), adult resting bone marrow (ABM CD34⁺), G-CSF mobilized bone marrow (mBM CD34⁺), and neonatal umbilical cord blood (CB CD34⁺); G-CSF mobilized peripheral blood leukocytes (mPB leukocytes); CD34⁻ cells purified from mPB leukocytes (mPB CD34⁻), adult resting bone marrow (ABM CD34⁻), G-CSF mobilized bone marrow (mBM CD34⁻), and neonatal umbilical cord blood (CB CD34⁻); CD14⁺ cells; and granulocytes. Transformed human cell lines included K526, an erythroleukemia; HL60, an acute promyelocytic leukemia; Jurkat, a T cell leukemia; HEK 293, epithelial cells from embryonic kidney transformed with adenovirus 5 DNA; and Hep3B hepatocellular liver carcinoma cells cultured in normal (HepB normal) or reduced oxygen tension (Hep3B hypoxia), or mock stimulated or stimulated with TGF- β .

Probes were designed by PrimerExpress software (PE Biosystems) based on the h16445 sequence. The h16445 sequence probe was labeled using FAM (6-

carboxyfluorescein), and the β 2-microglobulin reference probe was labeled with a different fluorescent dye, VIC. The differential labeling of the target sequence and internal reference gene thus enabled measurement in the same well. Forward and reverse primers and the probes for both β 2-microglobulin and the target h16445 sequence were added to the TaqMan[®] Universal PCR Master Mix (PE Applied Biosystems). Although the final concentration of primer and probe could vary, each was internally consistent within a given experiment. A typical experiment contained 200 nM of forward and reverse primers plus 100 nM probe for β -2 microglobulin and 600 nM forward and reverse primers plus 200 nM probe for the target h16445 sequence. TaqMan[®] matrix experiments were carried out on an ABI PRISM 7700 Sequence Detection System (PE Applied Biosystems). The thermal cycler conditions were as follows: hold for 2 min at 50°C and 10 min at 95°C, followed by two-step PCR for 40 cycles of 95°C for 15 sec followed by 60°C for 1 min.

The following method was used to quantitatively calculate h16445 expression in the various tissues relative to β -2 microglobulin expression in the same tissue. The threshold cycle (Ct) value is defined as the cycle at which a statistically significant increase in fluorescence is detected. A lower Ct value is indicative of a higher mRNA concentration. The Ct value of the h16445 sequence is normalized by subtracting the Ct value of the β -2 microglobulin gene to obtain a Δ Ct value using the following formula: Δ Ct = Ct_{h15571} - Ct _{β -2 microglobulin}. Expression is then calibrated against a cDNA sample showing a comparatively low level of expression of the h16445 sequence. The Δ Ct value for the calibrator sample is then subtracted from Δ Ct for each tissue sample according to the following formula: $\Delta\Delta$ Ct = Δ Ct_{sample} - Δ Ct_{calibrator}. Relative expression is then calculated using the arithmetic formula given by $2^{-\Delta\Delta$ Ct}. Expression of the target h16445 sequence in each of the tissues tested was then graphically represented as discussed in more detail below.

Figure 2 shows expression of h16445 as determined in a broad panel of tissues and cell lines as described above, relative to expression in Hep3B hypoxia cells. The results indicate significant expression in HL60 and K562 cell lines, skeletal muscle, activated and resting B and CD8⁺ cells.

Expression of h16445 was also detected by *in situ* hybridization of riboprobes to cellular mRNAs in the following human tissues: spleen, tonsil, lymph node, and colon (normal and inflammatory bowel disease). Sense and anti-sense riboprobes (RNA transcripts) of cDNA encoding h16445 were generated using ³⁵S-dUTP, T3, and T7 polymerases, and standard *in vitro* transcription reaction reagents.

Six µm sections of cryopreserved human tissue were prepared using a cryostat and annealed to glass slides, pre-hybed and hybridized to sense- and anti-sense h16445 riboprobes according to standard protocols. Slides containing hybridized tissues and riboprobes were washed extensively (according to standard procedures), dipped in NTB-2 photoemulsion, and were allowed to expose for two weeks. Slides were developed and counter-stained with Hematoxylin and Eosin to assist in identifying different subtypes of leukocytes. Data were recorded as pictures of these tissue sections as visualized under a microscope using bright and dark fields. The data from two separate experiments are summarized in Tables I and II below.

High levels of h16445 expression were detected in tonsil and thymus tissue. Tissue from normal and diseased colon (inflammatory bowel disease (IBD)), and some diseased synovium (rheumatoid arthritis) was shown to express intermediate levels of h16445. Very low to no expression of h16445 was detected in tissue from lymph nodes. No expression of h16445 was detected in spleen or normal synovium and some diseased synovium tissues (osteoarthritis).

Table 1. Expression Analysis of Human 16445 by *In Situ* Hybridization

Tissue	16445	Comments
Tonsil (PIT202)	++	Expression in follicles, high in germinal center, low in corona. No expression in interfollicular areas.
Tonsil (PIT221)	++	Expression in follicles, high in germinal center, low in corona. No expression in interfollicular areas.
Normal Colon (NDR39)	+	Expression in follicles, high in germinal center, low in corona. No expression in lamina propria. High background in eosinophils.
IBD (WUM02)	+	Expression in follicles, high in germinal center, low in corona. No expression in lamina propria. High background in eosinophils.
IBD (WUM04)	+	Expression in follicles, high in germinal center, low in corona. . No expression in lamina propria. High background in eosinophils.
IBD (WUM06)	+	Expression in follicles, high in germinal center, low in corona. No expression in lamina propria. High background in eosinophils.
Spleen (PIT268)	-	No expression.
Lymph Node (CLN484)	-	No expression of 16445. High expression of beta-actin.
Lymph Node (CLN820)	-	No expression of 16445. High expression of beta-actin.

Table II. Expression Analysis of Human 16445 by *In Situ* Hybridization

Tissue	16445	Comments
Tonsil (PIT222)	++	High expression in germinal centers and intermediate expression in medulla. No expression in interfollicular areas.
IBD (WUM2)	+	Expression in follicles, high in germinal center, low in corona. No expression in lamina propria. High background in eosinophils.
IBD (WUM4)	+	Expression in follicles, high in germinal center, low in corona. No expression in lamina propria. High background in eosinophils.
IBD (WUM6)	+	Expression in follicles, high in germinal center, low in corona. No expression in lamina propria. High background in eosinophils.
Thymus (BWH5)	++	No expression in cortex (immature thymocytes) and intermediate level of expression in the medulla, (mature thymocytes, although macrophages and dendritic cells also exist in medulla).
Lymph Node (CLN484)	+	Low expression in a small percentage (1%) of mononuclear cells in follicles.
Normal Synovium (NDR738a)	-	Very clean – virtually no infiltrate.
OA Synovium (NDR740c)	-	Extremely small sample – virtually no infiltrate.
RA Synovium (NEB02)	+	Low expression by a minority of mononuclear leukocytes in synovium.
RA Synovium (NEB03)	+	Low expression by a minority of mononuclear leukocytes in synovium.
RA Synovium (NEB04)	+/-	Little infiltrate in this sample. Interpretation difficult due to particulate matter.

Example 3: Functional Analysis of Human 16445 Signal Transduction

Amino acid sequence comparisons suggest that h16445 is a member of the hematopoietic Type I cytokine receptor family. Included in this family are the IL-2 receptor gamma chain dependent receptors for the cytokines IL-2, IL-4, IL-7, IL-9, and IL-15. In addition, these receptor-ligand complexes utilize common signaling intermediates such as the JAK and STAT family of proteins.

The signaling of h16445 was investigated as described below. Human 16445 was ectopically expressed in the human hepatoma cell line, HepG2. In this approach, HepG2 cells were cotransfected with cDNA clones encoding cytokine receptor subunits and a chloramphenicol acetyl transferase (CAT)-reporter plasmid containing eight copies of the cytokine-inducible hemopoietin receptor response element (HRRE-CAT) (Zeigler *et al.* (1995) *Eur. J. Immunol.* 25:399 and Morella (1995) *JBC* 270:8298). The ability of the transfected cells to signal when treated with the appropriate cytokine is measured by increased CAT activity. In addition, a plasmid encoding STAT5 was added to provide adequate signaling through this intermediate.

To test the effect of h16445 expression on signal transduction through known cytokine receptors, cotransfections were performed with HRRE-CAT, STAT5, and each of the receptor subunits IL-2 β , IL-4 α , IL-9 α , IL-7 α or TSLPR. In the case of the IL-4 and IL-9 receptors, the IL-2 receptor gamma chain was also introduced into cells. The effect of h16445 expression on signal transduction by the above-mentioned cytokine receptors was measured as a difference between cytokine-stimulated CAT expression in the presence or absence of h16445. Cytokine-stimulated expression was measured in cells transfected with control plasmid, 0.2 μ g/ml h16445 plasmid, or 1.0 μ g/ml h16445 plasmid 24 hours subsequent to the addition of cytokine ligand (Figure 3A and 3B).

Receptor signaling by IL-7R and TSLPR was the most dramatically affected by h16445 expression (Figure 3A and 3B). In cells transfected with 1 μ g/ml h16445 plasmid, IL-7- and TSLP-induced CAT activity was reduced by approximately 95% relative to cells transfected with control plasmid. Signal transduction through the other cytokine/cytokine receptor pairs was affected to a lesser degree. Signaling through IL-9/IL-9R was reduced by about 90% and signaling by IL-4/IL-4R was reduced by about

30%. Human 16445 expression had only a modest effect on signaling by the IL-2/IL-2R receptor pair.

An additional experiment was performed to determine if the cytoplasmic domain of h16445 can function as a hematopoietic Type I cytokine receptor, by producing a signal that induces a transcriptional event common to this family of receptors. In this experiment, a construct was prepared in which the cytoplasmic portion of h16445 was fused to the extracellular and transmembrane domains of the IL-9 receptor alpha chain. The resulting chimeric receptor construct was transfected into HepG2 cells alone or in combination with the IL-2 receptor gamma. Cytokine-induced CAT expression was measured as described above (Figure 4). Addition of IL-9 to cells transfected with the IL-9/h16445 receptor chimera and IL-2R γ resulted in a greater than 30-fold increase in CAT expression. These data demonstrate that the cytoplasmic domain of h16445 functions as a Type I cytokine receptor.

Example 4: Preparation of Monoclonal Antibodies Specific for h16445

An h16445-human IgG1Fc fusion construct consisting of the leader sequence from human CD5 plus the region coding for amino acids 1 to 234 of h16445 was prepared. Fusion protein was expressed from mammalian COS cells by transient transfection of the fusion construct using Lipofectamine (Gibco BRL) as per manufacture's instructions. Supernatants were harvested on day 3 and day 7. Fusion protein was purified using ProsepTM Protein G glass beads.

Balb/c mice were immunized with DNA encoding the fusion protein described above using gene gun delivery of DNA as described in Kilpatrick *et al.* (1998) *Hybridoma* 17(6). A serum titer could be detected against the h16445-human IgG1Fc fusion protein by ELISA (Enzyme Linked ImmunoSorbent Assay) using standard methodology (See Harlow and Lane, *Antibodies : A Laboratory Manual* (Cold Spring Harbor Laboratory Press); Coligan *et al.*, eds.; *Current Protocols in Immunology* (John Wiley & Sons).

Mice were boosted with h16445-hIgG1Fc fusion protein intravenously 4 days prior to harvesting of the spleens. Fusions were carried out using standard protocols. Spleen cells from one mouse were fused with SP2/0 myeloma cells using standard

polyethylene glycol (PEG) protocol (Harlow and Lane, *Antibodies : A Laboratory Manual* (Cold Spring Harbor Laboratory Press); Coligan et al., eds., *Current Protocols in Immunology* (John Wiley & Sons)).

- 5 Hybridoma lines were screened for secretion of h16445-specific antibodies using the following methods:
- 1) ELISA using plate bound h16445-human IgG1Fc or human IgG1 (Sigma).
 - 2) Transient transfection of COS or HEK293 cells with a plasmid encoding amino acids
10 1 to 234 of the extracellular domain of h16445 plus a His tag and the C terminal signal sequence from human placental alkaline phosphatase (GPI linker signal) or with control vector. Hybridoma supernatants were screened by FACs analysis for binding to cell-surface expressed h16445-His.
 - 3) Transient transfection of HEK293 cells with full-length h16445/pCDNA3.1 plasmid.
15 Hybridoma supernatants were tested for their ability to bind to the cell surface expressed full length h16445 by FACs analysis.
 - 4) Hybridoma supernatants were ranked according to their affinity for solid phase h16445 or human IgG1 using Biacore.
- 20 Screening of hybridoma cell lines by ELISA using the 16445-IgG1 fusion protein identified 27 positive supernatants. Of these all but one were also positive when screened by FACS analysis for binding to the GPI-linked h16445 extracellular domain expressed on COS cells. The cell lines were then similarly tested for binding to full-length h16445 expressed in HEK293 cells. All but two of these cell lines were also positive, although to
25 a lesser degree than observed for binding to GPI-linked h16445 extracellular domain expressed on COS cells. Representative experiments for h16445-GPI transfected HEK293 cells (h16445-EC HEK) and h16445 full-length transfected HEK293 cells (h16445-FL HEK) are shown in Figure 5 (A-D). Relative fluorescence intensity exhibited by these transfected cells tagged with particular hybridoma supernatants (peak
30 2) is shown versus that exhibited by untransfected cells (represented by peak 1), which

served as a control in these experiments. Similar positive binding results were also observed for the BiaCore analysis.

Hybridoma cell lines selected for their ability to secrete h16445 specific antibodies were cloned using ClonalCell™-HY Medium D (StemCell Technologies Inc) as per manufacturer's instructions.

Example 5: Phenotypic Analysis of h16445 Expression on Tonsilar Lymphocytes

Surgical specimens from tonsillectomy patients were manually dispersed in RPMI-1640 plus 0.5% BSA. Lymphocytes were purified by standard ficoll centrifugation using lymphoprep (Sigma). Nonspecific binding to Fc receptors was blocked using human Ig (Pharmingen). Cells were stained using supernatants from hybridomas secreting antibodies that recognized h16445-transfected HEK293 cells. 50 µL of each supernatant was incubated with 1×10^6 cells for 30 minutes on ice and then washed with RPMI-1640 plus 0.5% BSA. Cells were resuspended in RPMI-1640 plus 0.5% BSA containing PE labeled goat anti-mouse F(ab')₂ secondary antibody and incubated with cells for 30 minutes on ice and then washed twice in RPMI-1640 plus 0.5% BSA. For the final staining step, cells were incubated in CyChrome conjugated mouse anti-hCD19 and FITC conjugated mouse anti-hCD3 for 20 minutes on ice. Binding was then quantified using standard three-color analysis with a FACScan (Becton Dickinson) flow cytometer.

Staining was observed for all antibody supernatants that were positive for h16445-transfected cells. All expression was restricted to the CD19+ population with very few cells staining the CD3+ population, indicating primary expression in the B-cell compartment. Data was then analyzed by gating on the CD19+ population and comparing to staining using a control supernatant. Representative staining for three of the h16445-specific supernatants is shown in Figure 6. Expression of h16445 as detected by staining with the h16445-specific hybridoma supernatants (peak 2) is shown relative to that detected by staining with an irrelevant antibody supernatant specific for the chemokine neurotactin (Nt) (peak 1). Thus CD19 and h16445 staining were nearly coincident in this tissue sample. h16445 may thus represent a novel marker for B lymphocytes.

All publications and patent applications mentioned in the specification are indicative of the level of those skilled in the art to which this invention pertains. All publications and patent applications are herein incorporated by reference to the same extent as if each individual publication or patent application was specifically and

5 individually indicated to be incorporated by reference.

Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments of the invention described herein. Such equivalents are intended to be encompassed by the following claims.

10

11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65
66
67
68
69
70
71
72
73
74
75
76
77
78
79
80
81
82
83
84
85
86
87
88
89
90
91
92
93
94
95
96
97
98
99
100
101
102
103
104
105
106
107
108
109
110
111
112
113
114
115
116
117
118
119
120
121
122
123
124
125
126
127
128
129
130
131
132
133
134
135
136
137
138
139
140
141
142
143
144
145
146
147
148
149
150
151
152
153
154
155
156
157
158
159
160
161
162
163
164
165
166
167
168
169
170
171
172
173
174
175
176
177
178
179
180
181
182
183
184
185
186
187
188
189
190
191
192
193
194
195
196
197
198
199
200
201
202
203
204
205
206
207
208
209
210
211
212
213
214
215
216
217
218
219
220
221
222
223
224
225
226
227
228
229
230
231
232
233
234
235
236
237
238
239
240
241
242
243
244
245
246
247
248
249
250
251
252
253
254
255
256
257
258
259
260
261
262
263
264
265
266
267
268
269
270
271
272
273
274
275
276
277
278
279
280
281
282
283
284
285
286
287
288
289
290
291
292
293
294
295
296
297
298
299
300
301
302
303
304
305
306
307
308
309
310
311
312
313
314
315
316
317
318
319
320
321
322
323
324
325
326
327
328
329
330
331
332
333
334
335
336
337
338
339
340
341
342
343
344
345
346
347
348
349
350
351
352
353
354
355
356
357
358
359
360
361
362
363
364
365
366
367
368
369
370
371
372
373
374
375
376
377
378
379
380
381
382
383
384
385
386
387
388
389
390
391
392
393
394
395
396
397
398
399
400
401
402
403
404
405
406
407
408
409
410
411
412
413
414
415
416
417
418
419
420
421
422
423
424
425
426
427
428
429
430
431
432
433
434
435
436
437
438
439
440
441
442
443
444
445
446
447
448
449
450
451
452
453
454
455
456
457
458
459
460
461
462
463
464
465
466
467
468
469
470
471
472
473
474
475
476
477
478
479
480
481
482
483
484
485
486
487
488
489
490
491
492
493
494
495
496
497
498
499
500
501
502
503
504
505
506
507
508
509
510
511
512
513
514
515
516
517
518
519
520
521
522
523
524
525
526
527
528
529
530
531
532
533
534
535
536
537
538
539
540
541
542
543
544
545
546
547
548
549
550
551
552
553
554
555
556
557
558
559
560
561
562
563
564
565
566
567
568
569
570
571
572
573
574
575
576
577
578
579
580
581
582
583
584
585
586
587
588
589
590
591
592
593
594
595
596
597
598
599
600
601
602
603
604
605
606
607
608
609
610
611
612
613
614
615
616
617
618
619
620
621
622
623
624
625
626
627
628
629
630
631
632
633
634
635
636
637
638
639
640
641
642
643
644
645
646
647
648
649
650
651
652
653
654
655
656
657
658
659
660
661
662
663
664
665
666
667
668
669
670
671
672
673
674
675
676
677
678
679
680
681
682
683
684
685
686
687
688
689
690
691
692
693
694
695
696
697
698
699
700
701
702
703
704
705
706
707
708
709
710
711
712
713
714
715
716
717
718
719
720
721
722
723
724
725
726
727
728
729
730
731
732
733
734
735
736
737
738
739
740
741
742
743
744
745
746
747
748
749
750
751
752
753
754
755
756
757
758
759
760
761
762
763
764
765
766
767
768
769
770
771
772
773
774
775
776
777
778
779
780
781
782
783
784
785
786
787
788
789
790
791
792
793
794
795
796
797
798
799
800
801
802
803
804
805
806
807
808
809
810
811
812
813
814
815
816
817
818
819
820
821
822
823
824
825
826
827
828
829
830
831
832
833
834
835
836
837
838
839
840
841
842
843
844
845
846
847
848
849
850
851
852
853
854
855
856
857
858
859
860
861
862
863
864
865
866
867
868
869
870
871
872
873
874
875
876
877
878
879
880
881
882
883
884
885
886
887
888
889
890
891
892
893
894
895
896
897
898
899
900
901
902
903
904
905
906
907
908
909
910
911
912
913
914
915
916
917
918
919
920
921
922
923
924
925
926
927
928
929
930
931
932
933
934
935
936
937
938
939
940
941
942
943
944
945
946
947
948
949
950
951
952
953
954
955
956
957
958
959
960
961
962
963
964
965
966
967
968
969
970
971
972
973
974
975
976
977
978
979
980
981
982
983
984
985
986
987
988
989
990
991
992
993
994
995
996
997
998
999
1000